The Journal of Laboratory and Clinical Medicine

VOL. VIII ST. LOUIS, MO., APRIL, 1923 NO. 7

ORIGINAL ARTICLES

WHITE MICE AND THE ASSAY OF INSULIN*

PRELIMINARY REPORT

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THE physiologic assay of insulin presents problems of considerable complexity and at the same time problems of moment, since they involve both the sale and the clinical use of the product. It is highly desirable in regard to the latter for the clinician to have assurance that the dose he wishes to administer to the patient may be gauged from the potency stamped on the vial. The manufacturer, on the other hand must have confidence that such potency bears a close approximation to accuracy in that the extract must be sold on the basis of "units."

It is very apparent that in an attempt to reduce the factor of the individual physiologic variation of the test animal to a minimum one must multiply the numbers of such animals very considerably. In the case of rabbits such a measure involves a very material increase in expenditure of time and adds to the cost of production. The unit of insulin is essentially $\frac{1}{5}$ of the amount in c.c. required within four hours to lower the normal blood sugar of a rabbit calculated on the basis of 2 Kg., to a point approximating 0.045 per cent at which point the animal usually manifests convulsions.[1] The correlation of this unit with "carbohydrate equivalent" as determined by clinical observation on carefully selected and controlled diabetic patients will require time. Indeed, one may perhaps not expect more than an approximation and not an exact correlation since the individual patients on whom the assay is being conducted may manifest a change in carbohydrate toler-

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Received for publication, March 12, 1923.

ance from time to time. In addition, a further complication factor is the occasional fall in potency of the extract owing to certain unknown factors causing deterioration. The use of depancreatized dogs for purposes of assay presents many difficulties and requires constant administration of relatively large amounts of extract.

In July, 1922, some experiments were carried out on mice in the hope that by their use a more rapid and less expensive method of assay might be evolved. At that time the extract was not available for extensive use, and the work was not resumed until October. It was found that mice were susceptible in a high degree to insulin. In the early experiments the extract was given intravenously. After considerable experimentation it was established that intraperitoneal injection was more rapid in its effects and acted in higher dilutions than by the intravenous route. Consequently the latter method was discontinued. Some inconsistent results obtained led one to suspect that the Ph. of the saline used in making the dilutions was a factor to be taken into consideration. Consequently, the saline used subsequently for all dilutions was buffered after the manner recommended by Alice C. Evans[2] and a Ph. value of 6.8 was adopted.

The white mice used were obtained from local dealers with no guarantee as to purity of stock. The average weight was about 18 gms., and when possible, such a weight was chosen for use. As a routine procedure it was found advisable to use starved mice. Mice starved for 24 hours and immediately given oats after injection manifest a very marked tolerance to the effect of insulin. The tolerance under these conditions is such that a mouse may withstand, without any evidence of reaction, a dose of one hundred times the amount which will produce convulsions in a mouse which has been starved 24 hours and which has not been fed during the period of the test. Unstarved mice have not shown as consistent results as starved. As a rule they are less sensitive. The length of starvation, after a good deal of experimentation, was chosen as 24 hours. It is necessary to inspect the test animals closely to determine whether or not some of the mice show signs of weakness. Those that are not normal are rejected as unsuitable for testing purposes. The diet previous to starvation consisted in a plentiful supply of carrots and oats. A period of a few days was allowed to elapse before the mice were used for a subsequent test. No evidence was found to show that repeated use of the same mice was inadvisable. No consistent relationship between the weight of the mice and the time of onset of convulsions was established.

The amount of the injected fluid was kept constant, namely, 0.25 c.c. A 26-gauge needle and 0.5 c.c. syringe calibrated in $\frac{1}{20}$ were used. Standard glassware was used throughout. Dilutions were made so that the following amounts (in c.c.) of insulin were contained in 0.25 c.c. buffered saline Ph. 6.8: 0.01, 0.0075, 0.005, 0.00375, 0.0025, 0.0015, 0.00075, 0.0005, 0.00025. Lower dilutions were occasionally used. All glassware and saline were sterile.

On intraperitoneal injection of insulin there is a latent period of about 20 minutes or longer before symptoms become manifest. During this period the activity of the mice is sometimes decreased but frequently remains nor-

mal. Those mice which receive the larger injections of insulin become less active and finally motionless. At the end of the latent period they frequently exhibit postural eccentricities, clinging to the top or the side of the wire cage, or with the nose pressed firmly through its mesh. Respiration is increased in rate and excursion. Two types of reaction ensue. In one type ataxia and paresis predominate, in the other, convulsions. At present these types of reaction are both considered characteristic. The ataxia is first manifested in the gait of the mouse. The back is somewhat arched, the belly held further from the table than normal, and the mouse moves with a waddling motion. The posterior limbs rapidly become paretic and are extended posteriorly and laterally. The condition may proceed to almost complete paralysis. Before the latter stage convulsions sometimes ensue either spontaneously or in response to a sudden stimulus. The second type of reaction is as a rule of sudden onset and frequently occurs spontaneously. One may, however, often anticipate its spontaneity and induce a seizure by a sudden stimulus. Dropping the mouse from a height of a few inches will induce convulsions in an animal which would otherwise probably not evidence a seizure for some five or ten minutes. The convulsions are clonic and as a rule violent. The head is markedly retracted. The seizure may last 20 seconds or longer and recur if dextrose is not given. To be regarded as a typical reaction in either type the animal must recover within five minutes after the intraperitoneal injection of 0.25 c.c. of 15 per cent dextrose in 0.9 per cent saline. If, however, the mouse is allowed to remain ataxic too long, recovery may not occur, or be long delayed. The period of observation after injection of the extract has been somewhat arbitrarily set at two hours. Three mice at least should be used for each dilution. The highest dilution at which a typical reaction is evidenced is regarded as the end point. The expression of this end point has been designated as the minimum effective dose (M.E.D.).

Owing to the somewhat unsatisfactory methods of assay on rabbits and the difficulty of correlating the carbohydrate equivalent obtained in the clinics, it has been found impossible to express definitely the results obtained with mice in terms of units. It would appear, however, that 0.0025 c.c. of insulin is approximately equivalent to 5 rabbit units per c.c., which in turn is the equivalent of between 1-1½ grams of carbohydrate as determined on patients with severe diabetes.[3] The most potent extract tested up to the present has been one that produced convulsions with 0.0005 c.c. of extract. On the basis of 0.0025 c.c. insulin being the equivalent with mice of 5 units when rabbits are used for assay one may calculate the potency of an extract from the M.E.D. Thus a M.E.D. of 0.0005 represents a potency of 25 units per c.c.; a M.E.D. of 0.0015 represents 10 units per c.c.

It sometimes happens that a mouse which has received a higher dilution, that is, less extract, will evidence convulsions before one which has received a lower dilution. This is, however, not the rule. Occasionally a mouse seems to be tolerant. By the use of three or more mice for each dilution this variability may be eliminated.

The method lends itself admirably to the testing of unconcentrated ex-

tracts. If 0.0025 c.c. approximates 5 units per c.c. and one obtains a typical reaction with say 1 c.c. of dilute extract, which amount may be safely injected without danger, one may assume that the potency is somewhat in the neighborhood of 5 units per 500 c.c. It has also been found a useful method in laboratory investigations where the amount of available material for assay is small.

CONCLUSIONS

1. Starved mice are very susceptible to the intraperitoneal injection of insulin.

2. Mice fed immediately after the injection of insulin are very tolerant to its effect.

3. The correlation of the unit as determined by the use of rabbits with the unit as determined by the use of mice has been attempted; 0.0025 c.c. is approximately the equivalent of 5 (rabbit) units per c.c.

REFERENCES

[1]The expression of the unit has been modified since the original publication appearing in the Am. Jour. Physiology, lxii, No. 1.
[2]Jour. of Infect. Dis., 1922, xxx, 95.
[3]Personal communication, Graham, Campbell, Fletcher, Toronto General Hospital, Diabetic Clinic.

CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

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(Continued from page 369.)

Blood

INDICATIONS FOR EXAMINATION OF BLOOD

IN every disease of infancy and childhood no matter what the diagnosis, the patient's blood should be examined. In many cases, the examination may be limited to the cell elements and hemoglobin for which only a few drops of blood are required.

If congenital or acquired syphilis is suspected, a Wassermann test should be done on the blood. For this purpose, 3 to 5 c.c. of blood should be removed from the patient.

If diabetes is suspected, the blood sugar should be determined. The amount of blood required for this examination depends on the chemical method employed. For the Lewis-Benedict or the Folin-Wu method, 2 c.c. is required; for the Kowarsky method, 0.5 c.c. and for the Epstein method 0.2 c.c. suffice.

If nitrogen retention is suspected, the blood should be examined for the amount of nonprotein nitrogen for which 2 c.c. of blood is required. Some consider the uric acid determination as even more important than the nonprotein nitrogen. The amount of blood required varies between 2 to 4 c.c. depending on the method used. Urea nitrogen also requires 2 c.c. of blood. To throw light on the prognosis of nephritis creatinine should be determined. This examination requires 3 c.c. of blood. All constituents of nonprotein nitrogen of clinical importance may, however, be examined by taking only 5 c.c. of blood.

When acidosis is suspected, as in severe cases of diabetes, diarrhea or infections, the blood should be examined for the alkali reserve or its CO_2 combining power for which 0.5 to 1 c.c. of blood is required.

In rickets and tetany the calcium content of the blood may be examined; 3 to 5 c.c. being required for the examination.

Of some clinical importance is also the determination of cholesterol in diabetes for which examination 3 c.c. of blood is required and the determination of chlorides in nephritis with threatening edema for which examination 0.1 to 0.2 c.c. of blood is required.

METHODS OF OBTAINING BLOOD

When only a few drops of blood are required, as for the determination of the cell elements, hemoglobin and coagulation-time, the blood can be obtained by piercing the lobe of the ear or the finger with a sharp scalpel or

needle. When larger quantities of blood are required such as for the Wassermann test and blood chemistry, the blood may be obtained by making a large incision with scalpel in the patient's heel or by making a long incision in the intrascapular region and applying an old fashioned dry cup for withdrawing the blood. Both of these methods, although practiced by some physicians are, however, time-consuming and are unsatisfactory, especially for blood chemistry. In such cases, it is best to withdraw blood from some vein in the body.

In older children it may be possible to remove blood for examination from a vein on the arm or at the ankle. The technic is the same as that in adults which is as follows: The bend of the elbow or the internal surface of the ankle is selected as the site of puncture. A tourniquet (a soft rubber tube, a wide strip of gauze or even a handkerchief), is applied around the

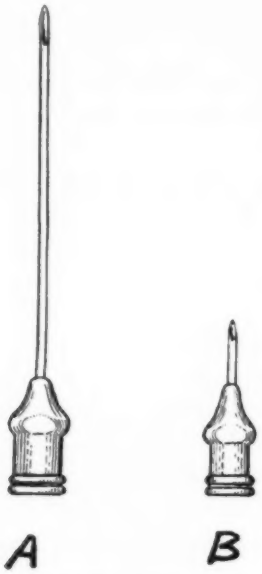

Fig. 10.—Needles employed for removal of blood from median or external jugular veins (*A*) and from the longitudinal sinus (*B*). Needles are shown in their actual size.

limb, 1 to 1½ inches above the point of puncture. The tourniquet should be tight enough to obstruct the venous, but not the arterial circulation. The limb is held by an assistant or is rested on the edge of a table and the area of operation is washed with alcohol and iodine. A 16- to 18-gauge needle, 1 to 1½ inches in length (Fig. 10*A*), fastened to a dry Record syringe is now introduced under the skin overlying the vein and pushed forward into the vein. The skin over the vein is preferably rolled between the thumb and index finger of the operator's left hand to facilitate the introduction of the needle into the vein. As soon as blood appears in the syringe, the plunger of the syringe should be withdrawn carefully until the desired amount of blood is obtained (Fig. 11). The tourniquet is now removed and a gauze bandage is applied around the limb over the area of puncture. No collodion is necessary.

In infants and young children where the veins are too small or where no veins can be distinguished on the skin, blood may be withdrawn from the jugular vein or from the longitudinal sinus.

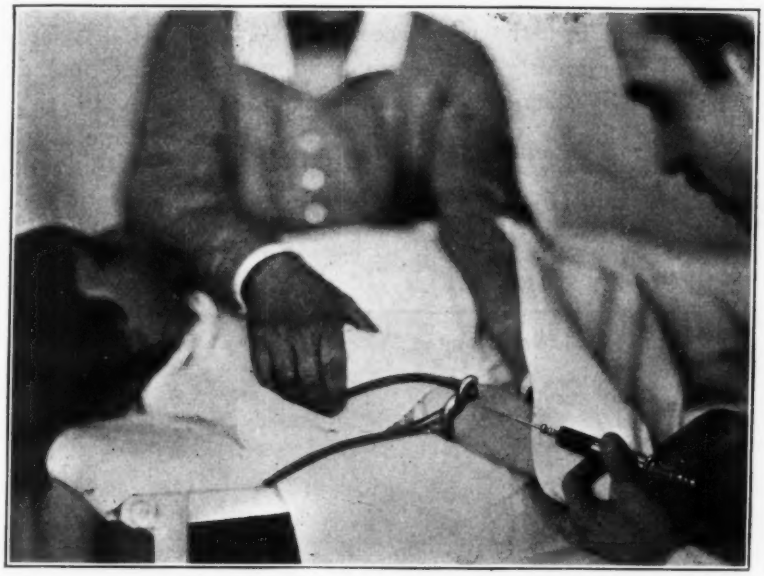

Fig. 11.—Removal of blood from median vein.

Fig. 12.—Removal of blood from external jugular vein.

JUGULAR VEIN PUNCTURE.—The child's arms are held closely to the trunk by a tightly wrapped sheet or blanket, the upper edge of which comes only to the shoulders. The child is placed on its back on a table with a small pillow or pad under the shoulders, allowing the head to fall slightly backwards. The chin is turned to the shoulder thus bringing the jugular vein into prominence and into the most accessible position. It may be made to stand out more prominently by the application of pressure just above the clavicle. The skin is cleaned with iodine and alcohol. A 16- to 18-gauge needle, 1 to 1½ inches in length, attached to a 10 c.c. syringe is introduced into the outstanding vein. The needle is introduced from above downward in a line with the vein (Fig. 12). It is best to pierce the needle through the skin only by the first thrust and then slowly introduce the needle into the vein.

Fig. 13.—Removal of blood from longitudinal sinus.

LONGITUDINAL SINUS PUNCTURE.—The child is placed in the recumbent posture with the head at the edge of table. It is best to envelop a sheet around child to prevent struggling. The hair over the anterior fontanelle is shaven and the scalp over this area is washed with alcohol and ether. A 16- to 18-gauge needle, ¼ to ½ inch in length (Fig. 10*B*), attached to a Luer syringe is now introduced at the lowermost portion of the posterior angle of the fontanelle, the needle pointing slightly backwards for a distance of ¼ to ⅜ of a cm. When the longitudinal sinus is reached, dark red blood appears in the syringe (Fig. 13). The desired amount of blood is now slowly withdrawn and collodion is applied to the puncture. If blood continues to ooze from the opening, the patient's head should be raised on a higher level than the rest of the body. If even this does not stop the bleeding, a tight bandage should be applied around the head.

PREVENTION OF CLOTTING.—For the determination of the cell elements, hemoglobin and Wassermann test, no precaution has to be taken to prevent the blood from clotting. For the chemical analysis of the blood it is important that the blood remain unclotted. For this purpose sodium citrate, potassium oxalate or lithium oxalate should be used, the two latter being preferable. In using potassium oxalate, 0.4 c.c. of a 2 per cent solution is employed for 5 c.c. of blood and 0.8 c.c. of a 2 per cent solution for 10 c.c. of blood. The oxalate solution is allowed to dry in the oven at the bottom of the bottle in which the blood is to be collected. A very simple way of preventing clotting of the blood is to run the oxalate solution through the needle and syringe before taking the blood. Even more simple is the usage of 2 to 3 crystals of potassium oxalate in the test tube in which the blood is to be collected. Lithium oxalate, which is considered the best anticoagulant, can be prepared only where good laboratory facilities are available.

CONTAINERS.—The blood should be collected directly from the needle in the vein or from the syringe into a wide-mouthed specimen bottle, 10 to 15 c.c. capacity. The receptacle should contain no water, for water tends to hemolyze the blood, a condition that affects the chemical reaction and makes for inaccuracies in the chemical reading. Constant shaking of the bottle is necessary while the blood is being collected so that the blood may be thoroughly mixed with the anticoagulant.

TIME ELEMENT.—For chemical examination blood should be withdrawn from the patient before breakfast, as food has a tendency to increase the concentration in the blood of the chemical substance to be examined. It has been found, for instance, that blood sugar increases right after the ingestion of food and that the increase continues from one to two hours. The same is true to a lesser degree of urea. The blood should be examined as soon as possible after it has been removed from the body, at least within twelve hours.

TABLE I

REMOVAL OF BLOOD FOR DIAGNOSTIC PURPOSES

DETERMINATION	AMOUNT NECESSARY	METHOD OF REMOVAL
Cell count Differential count	Few drops	Ear or toe by sharp scalpel
Coagulation	1 to 2 drops	" "
Wassermann test	3 to 5 c.c.	Cut in heel Needle in median vein (older children) Needle in jugular vein or longitudinal sinus (in infants)
Blood chemistry		
Nonprotein nitrogen 2 c.c. Urea nitrogen 2 c.c. Creatinine 3 c.c. Uric acid 2 c.c.	5 c.e. for determination of nonprotein nitrogen and its constituents	Same as for Wassermann except that an anticoagulant must be added
Sugar 0.2 to 2 c.e. Cholesterol 3 c.e. Chlorides 0.2 c.e.		
Alkali reserve 0.5 to 1 c.c.		Cut in heel or needle in vein
Blood culture 1 to 3 c.c.		Needle in vein
Blood matching 2 drops to 1 c.c.		Heel or vein
Fragility few drops to 2 c.c.		Heel or vein

TECHNIC OF CELL COUNTING.—

1. *Red Cell Count.*—After piercing the ear or finger with a sterilized needle or scalpel, blood is drawn up in the red blood cell pipette (Thomas Zeiss 1-101) to mark 0.5 and Hayem's solution (Mercuric Chloride 0.5 gm.; sodium sulphate 5.0 gm.; sodium chloride 2.0 gm.; distilled water 200 c.c.) to mark 101. The pipette is shaken and a drop is placed in the counting chamber. The cells contained in 100 small squares are counted and the number obtained is multiplied by 8000, or the cells in 80 squares are counted and the result multiplied by 10,000 by adding four zeros to the number obtained. This gives the red cell content per cm.

2. *White Cell Count.*—Blood is drawn up in the white cell counting chamber (1-11) to mark 0.5 and acetic acid (1.5 per cent) to mark 11. The cells in several squares are counted, the average for one square obtained and this number is multiplied by 200, to get the content of white cells per cm.

3. *Differential Cell Count.*—A drop of blood is spread on a slide with the edge of a cover-glass or of another slide or with cigarette paper. After the blood has dried in the air, the smear is covered with Wright stain for one minute followed by the addition of distilled water, drop by drop, for 4 or 5 minutes (depending on the strength of the Wright stain). The slide is then washed off with plain water, blotted and examined. At least 100 white cells should be counted and recorded. The red cells should have been noted for their shape, presence or absence of nuclei and for abnormal cells. The white cells should be counted according to their types (small and large lymphocytes, transitional, neutrophiles, eosinophiles and basophiles). Bacteria and parasites should also be noted.

INTERPRETATION OF CELL ELEMENTS IN BLOOD.—There are several important points to be kept in mind in connection with the determination of the cell elements in the blood of children.

TABLE II

EXAMPLE OF THE DISTRIBUTION OF THE CELL ELEMENTS IN THE BLOOD OF INFANTS AND CHILDREN.

AGE	HB.	RED	WHITE	N	SM.	LM.	T	E
3 days	95	7,500,000	17,200	49	34	15	12	
7 weeks	90	3,980,000	8,200	14	77	5	3	1
next day	90	4,100,000	9,800	21	70	4	5	
next day	80	4,000,000	9,860	11	81	3	3	2
3 months	85	4,300,000	10,000	12	67	12	5	1
6 months	80	4,400,000	12,200	22	63	13	2	0
8 months	75	4,200,000	11,500	37	41	18	3	1
3 years	90	4,600,000	13,200	46	38	4	6	6

The number of red blood cells is high in the newborn, usually reaching over 6,000,000 per cm. and is low during infancy and childhood averaging 4,000,000 per cm. No diagnosis of anemia should therefore be made in infants and children unless the red cells are markedly below 4,000,000 per cm. In newly born babies, especially those born prematurely, or those showing marked jaundice, nucleated red blood cells are commonly found. Later in

infancy and childhood, however, nucleated red cells indicate a pathologic condition.

The leucocytes in the newborn vary between 15,000 to 20,000 per cm., 60 to 70 per cent of the number being polymorphonuclear in type. From the second week on, extending all through infancy and childhood, the number of leucocytes varies between 10,000 and 15,000, with a preponderance of lymphocytes, the number of lymphocytes varying between 40 to 60 per cent. The eosinophiles vary between 1 and 3 per cent. Transitional cells range between 2 and 6 per cent except in the newborn when their number may be still higher (Table II.) The blood platelets vary between 250,000 to 300,000 per cm. all through infancy and childhood.

With these normal variations in mind, the structural examination of the blood in children assumes greater significance. A leucocytosis by itself means very little if anything, unless it is very high, such as 20,000 to 25,000. A leucocytosis with a relative neutrophilia indicates infection. Leucopenia is important because of its presence in measles, german measles, chicken pox, influenza and typhoid (Table III). It must be understood, however, that the leucopenia is present only in certain stages of these diseases, usually in the early stages, the blood changing to a leucocytosis toward the end of the disease or when complications take place.

TABLE III

LEUCOCYTIC CHANGES IN ACUTE INFECTIOUS DISEASES IN CHILDREN

Scarlet	Marked leucocytosis and neutrophilia, beginning with the prodromal symptoms, rising still higher on appearance of rash and gradually declining with improvement of the patient. Lymphocytes are diminished, increasing gradually with improvement. Eosinophiles are high during eruptive stage, at times reaching to 25 per cent.
Measles	Leucopenia and neutrophilia returning to normal when rash is fully developed.
German Measles	Leucopenia and relative lymphocytosis.
Chicken Pox	Leucopenia and leucocytosis.
Diphtheria	Leucocytosis and neutrophilia. Eosinophiles diminished or absent.
Erysipelas	Leucocytosis and neutrophilia in proportion to severity. Eosinophiles diminished or absent.
Mumps	Moderate leucocytosis, predominance of lymphocytes.
Grippe	Leucocytosis and neutrophilia.
Epidemic Influenza	Leucopenia and lymphocytosis early. Neutrophilic leucocytosis lasting several days on recovery or on onset of complication.
Pertussis	Leucocytosis and lymphocytosis.
Pneumonia	Marked leucocytosis and neutrophilia. Leucocytes ranging between 20,000 to 80,000; neutrophiles ranging between 70 to 98 per cent. Eosinophiles absent throughout disease but reappear on day of crisis.
Meningococcus Meningitis	Leucocytosis and neutrophilia.
Typhoid	Leucopenia and lymphocytosis. Eosinophiles diminished or absent. There is often a leucocytosis on the first day of the disease.

An eosinophilia over 4 per cent is present in eczema, asthma or intestinal parasites, reaching in some cases as high as 20 to 30 per cent. On the other hand, eosinophiles are absent in certain infectious diseases (Table III).

Arnett index and Opsonic index, at one time quite popular are not commonly employed now as a clinical aid. The same is true with inclusion bodies at one time considered specific for scarlet fever.

HEMOGLOBIN.—Hemoglobin is most easily determined by the Talquist hemoglobinometer, an instrument sufficiently accurate for clinical purposes.

At birth, the hemoglobin is usually over 100 per cent compared to the adult standard. During infancy, however, the hemoglobin is usually below that of the normal adult. During the school age, most children present a marked anemia spoken of in the literature as "school anemia." This possibly results from the overexertion and fatigue to which many modern school children are subjected. No diagnosis of organic anemia or disease of the blood should be made on the mere lowering of hemoglobin. One must take into consideration the number and type of cells, the hemoglobin and the color index (Table IV).

TABLE IV

BLOOD FINDINGS IN ANEMIAS OF INFANTS AND CHILDREN

TYPE	RED CELLS	HB.	COLOR INDEX	WHITE CELLS	TYPE OF WHITE BLOOD CELLS	ABNORMAL CELLS
Secondary anemia	Diminished according to degree of anemia	Diminished	1 or below	Slightly increased	Lymphocytes relatively increased	Poikilocytosis
Von Jaksch anemia	Diminished; 2,000,000 or less	Diminished to 20-25%	Normal or below	Increased; 25,000 to 60,000	Mono- or polymorphonuclear Eosinophiles increased	Nucleated red; mainly normoblasts; in severe cases megaloblasts
Acute lymphatic leukemia	Diminished; 2,000,000 to 1,000,000	20 to 30%	1 or below	Greatly increased; 50,000-100,000	Lymphocytosis 90-98%; chiefly large lymphocytes	
Chronic lymphatic leukemia	Diminished; 2,000,000 to 1,000,000		1 or below		Lymphocytosis; chiefly small lymphocytes	
Splenomyelogenous leukemia	Diminished; 2,000,000 to 1,000,000	Diminished; 20 to 30%	1 or below	50,000 to 200,000	Neutrophiles relatively increased Eosinophiles increased	Myelocytes and mast cells in large numbers
Chlorosis	Diminished; 3,500,000 to 2,500,000	35 to 40%	1 or below	Normal	Lymphocytes relatively increased	Poikilocytosis
Pernicious anemia	Greatly diminished. May fall to less than 1,000,000	Diminished as low as 20%	Above 1	Markedly diminished	Lymphocytes relatively increased	Nucleated red; more megaloblasts than normoblasts; Poikilocytosis

COAGULATION.—Coagulation of blood is both an interesting and important phenomenon. It is interesting to see a body tissue that is fluid while in the body coagulate as soon as it leaves the body. Normally blood coagu-

lates in 5 to 10 minutes after it leaves the body. Jaundice, sepsis, syphilis and hemophilia retard the coagulation of blood. In the first three diseases, the delay of coagulation is due to the hemolytic processes going on in the blood.

The following table from Ottenberg shows the importance of coagulation in differential diagnosis (Table V). Of utmost importance is the determination of coagulation time in surgery. No operation should be performed on a child without previous determination of the coagulation time of the patient's blood.

TABLE V

COAGULATION AS AN AID IN DIFFERENTIAL DIAGNOSIS OF HEMORRHAGIC DISEASES IN CHILDREN

	COAGULATION	BLEEDING TIME	PLATELETS
Hemophilia	Greatly prolonged	Normal or plus	Normal
Hemorrhage of newborn	Greatly prolonged	Prolonged	
Purpura hemorrhagica	Normal	Greatly prolonged	Greatly decreased
Purpura, secondary to			
1. Jaundice	Prolonged		
2. Chloroform	Prolonged		
3. Scurvy	Normal	Normal	Normal
Purpura simplex Arthritic purpura Visceral purpura	Normal	Normal	Normal

The mechanism of hemophilia is still unknown. It has been found, however, that there are two types of hemophilia; one due to calcipriva, and another due to thrombopriva. In the first type of cases, the calcium in the blood is diminished, and in the other type, the calcium in the blood is normal, but the amount of thrombin is lessened.

Therapeutically, it is often desirable to prevent coagulation of the blood. This is most important in cases where large quantities of blood have to be transfused into a child. Two-tenths per cent sodium citrate has been found useful in preventing coagulation.

BLOOD CULTURE.—A positive blood culture is of great assistance in diagnosis as it ascertains the causative organism, providing it is known that the culture media was not contaminated. A negative blood culture does not exclude the presence of bacterial organisms in the blood. Blood culture is usually most positive early in the disease. This is especially true in typhoid and pneumonia. It is found to be most positive when the patient has a high temperature.

Technic.—Three to 5 c.c. of blood is withdrawn from the median or jugular vein or from the longitudinal sinus, by means of a syringe (method described above). The blood is introduced into a flask containing 100 or 200 c.c. of sterile glucose broth. This is incubated for 24 hours and examined for the respective bacteria. The culture should be returned to the incubator and examined at intervals of 24 hours for a period of one week. This should be done in order to detect the more slowly growing organisms occasionally encountered, such as a poorly growing streptococcus viridans.

AGGLUTINATION.—When blood serum agglutinates a certain bacterial organism, it may be taken as evidence that the body is invaded by that particular organism.

Agglutination is mainly used in the diagnosis of typhoid, paratyphoid and dysentery. The agglutination test for typhoid is known as the Widal test. The technic is as follows:

A drop of blood serum or dried blood is diluted with 24 drops of normal salt solution. A drop of suspension of typhoid bacilli, which may be obtained from commercial laboratories, is now added to the diluted blood and examined on a hang-drop slide or on a flat slide. If the patient suffers from typhoid fever, the typhoid bacilli clump and if living bacilli are used, they become motionless in a few minutes.

The reaction may be obtained macroscopically by adding typhoid organisms to diluted blood serum and allowing to incubate overnight.

The Widal test is usually negative during the first week of the disease but becomes positive at the beginning of the second week and remains positive for months and years. A positive Widal, therefore, means that the patient either has or has had typhoid.

Pneumococcus agglutination is used to determine the type of pneumococcus invading the body in question after the organism has been isolated from the blood. If Type I is found, serum may be given to the patient. Type III is considered fatal.

BLOOD MATCHING.—Blood transfusion is used very frequently in pediatric practice in cases of sudden or gradual loss of blood. It has been found that the serum of a small percentage of individuals will clump the red blood cells of certain other individuals. In order to avoid any accidents, it is necessary to group or match the blood of the donor and of the recipient to ascertain that the serum of the patient would not agglutinate the corpuscles of the donor and *vice versa*. A number of methods have been described for blood grouping and blood matching. For practical purposes, the method of Lee or that of Kimpton will suffice.

Method of Lee.—A small amount of blood is collected from a patient (1 c.c. from the ear or finger is sufficient) and allowed to clot. The serum is then obtained. One drop of this serum is placed on a slide and mixed with a drop of a suspension of blood of the donor taken into 1.5 per cent citrate solution. (A few drops of blood are taken into approximately ten times the amount of 1.5 citrate solution and shaken. It is very important that the blood be dropped directly into the citrate, and should not be coagulated partially.) The result will appear in a few moments and is best examined under the microscope, where in the event of a positive test, marked agglutination will be evident. The reaction will also be evident macroscopically. In the event of a negative test it is a wise precaution to raise the cover-glass, and after making sure that the serum and cells are well mixed, to examine the preparation again. The only possible source of confusion is the appearance of rouleaux of the red corpuscles, indicating too thick an emulsion. If the test is negative, transfusion may be regarded as entirely safe.

Method of Kimpton.—Two to three drops of the donor's blood is collected into a small tube containing 0.5 per cent oxalate or salt solution (Solution 1). The oxalate prevents coagulation, thus keeping the corpuscles free.

Two to three drops of the donor's blood is collected into 0.5 c.c. of distilled water (Solution 2). This destroys the red blood cells and preserves the serum.

The above procedures are repeated with the recipient's blood. (Solution 3 = corpuscles and Solution 4 = serum.)

A drop of the donor's corpuscles (Solution 1) is now placed on a hang drop slide with a drop of the recipient's serum (Solution 4). The procedure is repeated with the donor's serum (Solution 2) and the recipient's corpuscles (Solution 3). The slide is observed under the microscope for clumping. Often clumping may be observed even with the naked eye. If clumping is present, the donor's blood cannot be used for transfusion.

Harp found that isoagglutination is rarely present at birth and during the first month of life. According to his findings, no test would have to be made on blood to be transfused to an infant during the first month of life.

Fragility of Blood Corpuscles.—Normally red blood cells do not hemolyze in salt solution above a dilution of 0.44 per cent. In hemolytic condition of the blood, the cells hemolyze with a higher salt solution even as high as 0.6 to 0.8 per cent,—in other words the cells are fragile. It is claimed that the type of jaundice can be differentiated by the fragility of the blood cells; hemolytic jaundice making the blood more fragile, obstructive jaundice producing no change in their resistance. The test cannot be considered specific, although it serves as corroborative evidence.

Wassermann Test.—Although a positive Wassermann test may be obtained occasionally in disease other than syphilis such as in leprosy, jaundice or even in the early stages of scarlet fever, a 4-plus positive Wassermann test should be taken as an indication of a luetic infection, providing the test has been done with more than one antigen. A doubtful positive test should always be repeated. A negative Wassermann test does not exclude syphilis as the infection may be too recent in origin, may have subsided under treatment or may be localized in the central nervous system. In the latter case the cerebrospinal fluid Wassermann may be positive in spite of the negative reaction in the blood.

Occasionally a Wassermann test is negative in cases where clinical symptoms point to congenital or acquired syphilis. In such cases the test may be made more effective by giving the child a dose of neosalvarsan or several mercuric rubs, and the Wassermann repeated. Such a test is called provocative Wassermann.

SAFETY OF LOCAL ANESTHETICS*

With Particular Reference to Cocaine and Butyn

By Carl Nielsen and John A. Higgins, Chicago, Ill.

AN IDEAL local anesthetic for use in the practice of medicine and dentistry is one which combines universal efficiency with clinical safety, and which is easily soluble, capable of sterilization by boiling, and nonirritating. The professions are familiar enough with the unpleasant experiences with one or the other of the local anesthetics of the so-called "cocaine-group" that have been in common use for years past. This applies particularly to cocaine itself which, in spite of its many dangers, used to be the local anesthetic of choice until equally or more efficient but less dangerous products were developed for the purpose. The progress of recent years in this direction has advanced to the point where the local anesthetic is chosen with preference from among the group of non-habit forming synthetics often termed "substitutes for cocaine"; while cocaine itself is being regarded as a dangerous local anesthetic to be used only when no other is available. Sollmann[1] classifies all these anesthetics under the general term, "cocaine-group." They are all alkamine esters of aromatic acids, but the synthetic ones differ from cocaine in that they are not habit forming.

About two years ago, a new synthetic local anesthetic was introduced and described by Kamm, Adams and Volwiler, 1920,[2] and by Volwiler, 1921;[3] namely, para-amino benzoyl gamma di-n-butyl amino-propanol hydrochloride. This, as well as the succinate of the same base, was investigated pharmacologically by Bonar and Sollmann.[4] Our experiments include in addition to these salts also the much more soluble sulphate (Butyn). These extensive pharmacologic tests and the subsequent clinical trials established the superior efficiency of butyn to cocaine and procaine.

The first question asked, when such a new local anesthetic is introduced, is naturally, "How toxic is it compared to these?" The physician or dentist usually expects an approximate comparative figure in answer to the question, since unfortunately it often is customary to express the toxicity of local anesthetics in such figures without explaining how they are obtained. Although it has been repeatedly demonstrated that the ratio of toxicity of each of these local anesthetics varies greatly in the different species of animals, and that the toxicity of one and the same anesthetic in the individual species varies according to the concentration and mode of application, yet these arbitrary figures are very often applied as a direct measure for the clinical safety of the anesthetic in human therapy.

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Received for publication, February 28, 1923.

Neither the efficiency nor the toxicity of a local anesthetic can be judged from a series of tests on a single animal species. Nor do the toxicity and side actions, as demonstrated on various laboratory animals, offer an approximate measure for the safety or non-safety in human therapy. The synthetic anesthetics, as a rule, present less variation and leave less uncertainty than cocaine in that direction.

TABLE I

SUBCUTANEOUS INJECTIONS IN ALBINO RATS

The solutions used contained 10% of the anesthetics and 0.004% of epinephrin:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	370	1
Butyn	190	2

TABLE II

SUBCUTANEOUS INJECTIONS IN RABBITS

The solutions used contained 5% of the anesthetics:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	200	1
Butyn	55	3-⅔

Bonar and Sollmann[4] in their work on new local anesthetics state: "The current methods of estimating efficiency, toxicity and irritation * * * may render considerable practical service by cutting short the career of distinctly unpromising anesthetics before they are introduced into clinical practice. The final selection of the more valuable from the crowd of the more or less promising local anesthetics is much more difficult. * * * Judgment must be cumulative since it cannot for the present be absolute." The safety of a "promising" local anesthetic can be judged only after long continued clinical experience with it.

We have, during the past five years, investigated a number of synthetic anesthetics prepared by Adams, Volwiler, Kamm and others in their endeavor to find one safer and more universally efficient than cocaine. Our work includes those investigated by Bonar and Sollmann; we also found butyn to be the most promising.

Bonar and Sollmann report the toxicity of para-amino benzoyl gamma di-n-butyl amino-propanol hydrochloride and cocaine as obtained by subcutaneous injections into albino rats and intravenous injections in cats and rabbits. Our investigations include aside from these the subcutaneous toxicity in rabbits, cats and dogs and the toxicity by stomach in cats; the action on blood pressure and respiration was also investigated. The following are extracts from our records, showing the vast differences in relative ratio of toxicity of these anesthetics, according to the animal used, and the mode of administration.

Similar series of tests were conducted with butyn solutions, previously steam sterilized for one hour or autoclaved half an hour at 15 pounds pressure. Neither the efficiency nor the toxicity was influenced by these pro-

cedures. In all these tables cocaine is used as a standard = 1 for the ratio of toxicity; it must be understood that the apparent variation in the ratios of toxicity of butyn expressed in this manner are due mainly to the great variation of toxicity of cocaine in these various animals.

This is conveniently expressed in the accompanying graphic illustration of the ratios of toxicity by the subcutaneous method. (Charts 1 and 2.)

It will be seen that butyn subcutaneously is twice as toxic on rats, 3⅔ times as toxic on rabbits, approximately equal in toxicity on cats, but slightly less toxic than cocaine on dogs. Introduced directly into the blood stream in rabbits and cats and orally to cats (Tables V, VI and VII) butyn is about

TABLE III

SUBCUTANEOUS INJECTIONS IN CATS

The solutions used contained 15% of the anesthetics:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	50	1
Butyn	55	1

TABLE IV

SUBCUTANEOUS INJECTIONS IN DOGS

The solutions used contained 15% of the anesthetics:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	45	1
Butyn	55	⅘

TABLE V

INTRAVENOUS INJECTIONS IN RABBITS

The solutions used contained 1% of the anesthetics and were injected at the rate of 1 c.c. in 18 seconds:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	12	1
Butyn	12	1

TABLE VI

INTRAVENOUS INJECTIONS IN CATS

Bonar and Sollmann report the results obtained by Hatcher with para-amino benzoyl gamma di-n-butyl amino-propanol hydrochloride, this salt being termed "H" (butyn is the sulphate of the same base).

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	15	1
"H"	15 (approximately)	1

TABLE VII

ORALLY IN CATS (ADMINISTERED IN GELATINE CAPSULES)

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	40	1
Butyn	45	8/9

equal in toxicity to cocaine. It is evident that we cannot answer the question of safety to the human in terms of "toxicity" to experimental animals, since we would be as justified in saying that butyn is equal in toxicity or less toxic than cocaine as we are in stating that it is twice as toxic. The results in the larger animals are as good—if not indeed a better—guide to our judgment. No final conclusion as to the safety of a local anesthetic of this character to the human and the consequent extent of its usefulness, must be drawn from toxicity tests on animals. Such tests are, of course, of great value as preliminary indications, but they are apt to be misleading if confined to one or two species of small experimental animals. This is particularly true as long as we use cocaine, which is so inconsistent in its toxic effect on various animals, as a standard.

Butyn, for example, has a uniform ratio of toxicity subcutaneously in

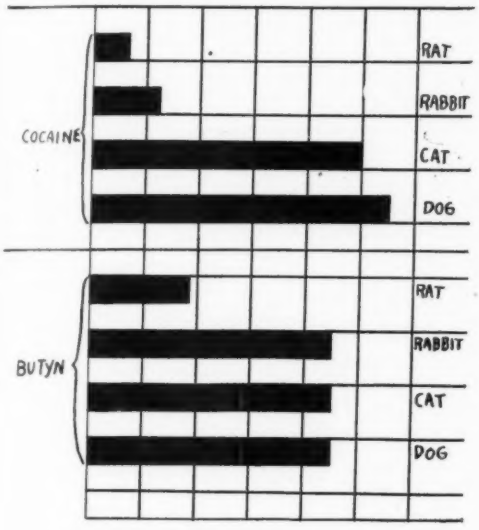

Chart 1.—Showing the comparative variation in toxicity of cocaine and butyn by subcutaneous injection in different animals.

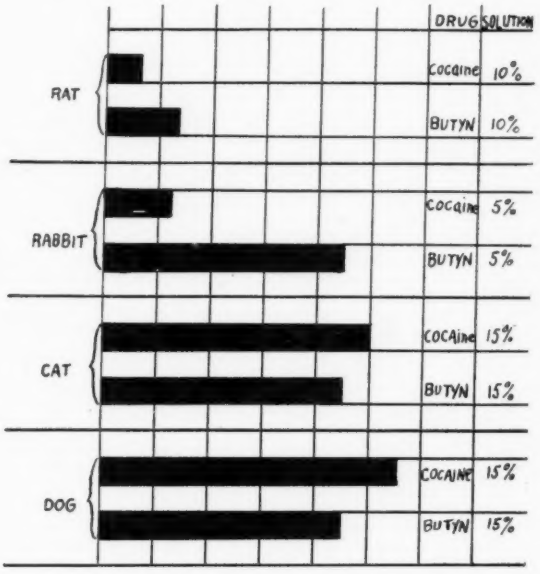

Chart 2.—Showing the comparative variation in toxicity of cocaine and butyn by subcutaneous injection in different animals.

rabbits, cats and dogs, while cocaine becomes more toxic in the same order, until in the dog it is more toxic than butyn. The toxicity of cocaine is more selective. Dixon[5] has shown that there is a definite relation between the amount of brain substance per weight of animal and the dose of cocaine required to produce convulsions. The convulsions are a result mainly of its action on the neurones of the brain. The higher the development of the brain, the more toxic its action. It requires 20 milligrams per kilogram bodyweight of cocaine to produce convulsions in the dog, but only 12 milligrams to produce a similar reaction in the ape. Our graphic cocaine chart is in conformity with Dixon's findings. In contrast, the butyn chart shows that the minimum fatal dose of this anesthetic is the same per kilogram animal in the rabbit, cat and dog. While cocaine, therefore, according to Dixon's observations, is more toxic to man than to animals, it is improbable that butyn would show great

variation—if any. The treacherous action of cocaine in the human on one hand and the degree of safety with which butyn has been extensively used clinically on the other, point in the same direction.[6, 7] So does the comparative action of the two on the blood pressure and respiration.

Figs. 1 and 2 show the effect of cocaine and butyn on the blood pressure and respiration in the same dog. The injections were 1 c.c. of a 1 per cent solution of each anesthetic with 30 minutes interval. Butyn was injected first. It will be seen that butyn caused only a very slight preliminary stimulation, then an abrupt fall for about a minute, followed immediately by a sharp rise to normal. There were no convulsions. Cocaine produced a considerable rise in pressure of short duration, then a gradual and long sustained

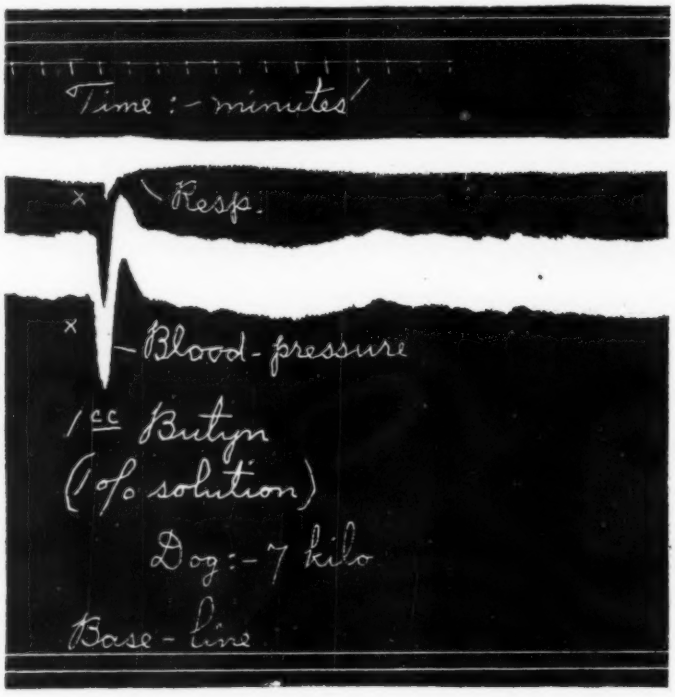

Fig. 1.

fall, accompanied by convulsions. Cocaine was also more depressing than butyn on the respiration.

In another dog (Fig. 3), the order of injections was reversed, the cocaine again showing a more prolonged depression. In a third and larger dog (Figs. 4 and 5) trichlorbutyl alcohol was used as an anesthetic and 5 c.c. of a 1 per cent solution of the local anesthetics were injected with a three hour interval. Again cocaine was more depressing than butyn. The reactions on this dog are not as distinct, probably due partly to the general anesthetic used. In comparing these tracings it should be considered that the amounts injected were the same for butyn and cocaine, although butyn as a local anesthetic, is at least twice as efficient as cocaine in all cases where these anesthetics are applied therapeutically.

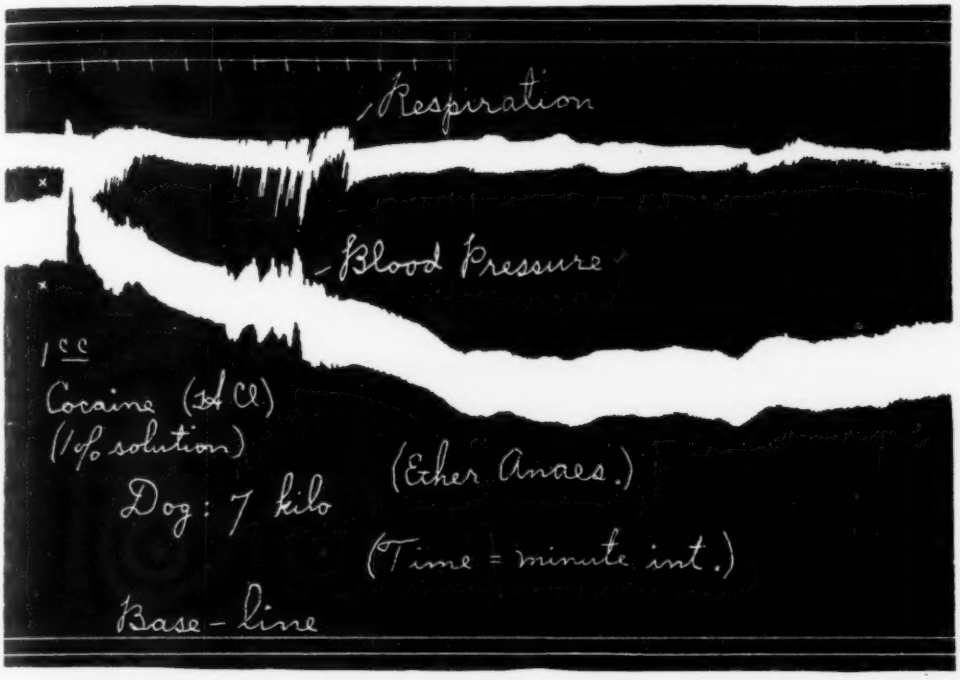
Respiration
Blood Pressure
1 cc
Cocaine (HCl)
(1% solution)
Dog: 7 kilo
(Ether Anaes.)
(Time = minute int.)
Base-line

Fig. 2.

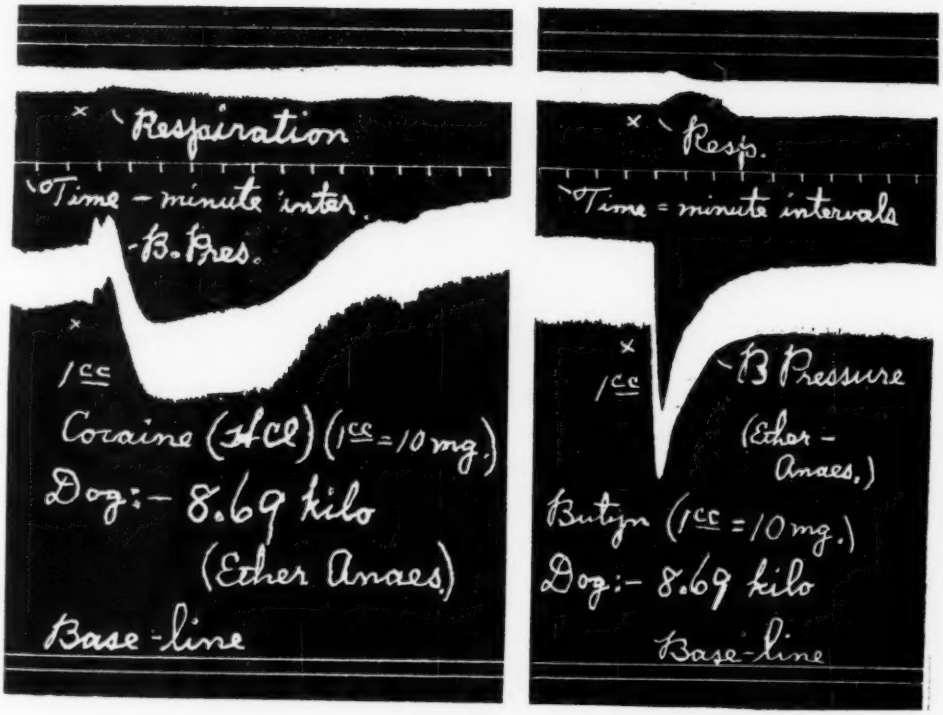
Respiration
Time - minute inter.
B. Pres.
1 cc
Cocaine (HCl) (1 cc = 10 mg.)
Dog:- 8.69 kilo
(Ether Anaes)
Base-line
Resp.
Time = minute intervals
1 cc
B Pressure
(Ether - Anaes.)
Butyn (1 cc = 10 mg.)
Dog:- 8.69 kilo
Base-line

Fig. 3.

Undesirable side actions in patients treated with local anesthetics of this character will occur. They have been too frequent with cocaine and similar cases have been reported even from the use of procaine. Such cases are of course not always directly referable to the anesthetic, and may even be purely psychic in character. Butyn cannot be expected to be an exception to this rule. Years of clinical experience are necessary for proper judgment.

THE USE OF DRUGS IN THE TREATMENT OF POISONING BY LOCAL ANESTHETICS

Several methods of treatment for poisoning by local anesthetics have been proposed, but none of them have been very satisfactory.

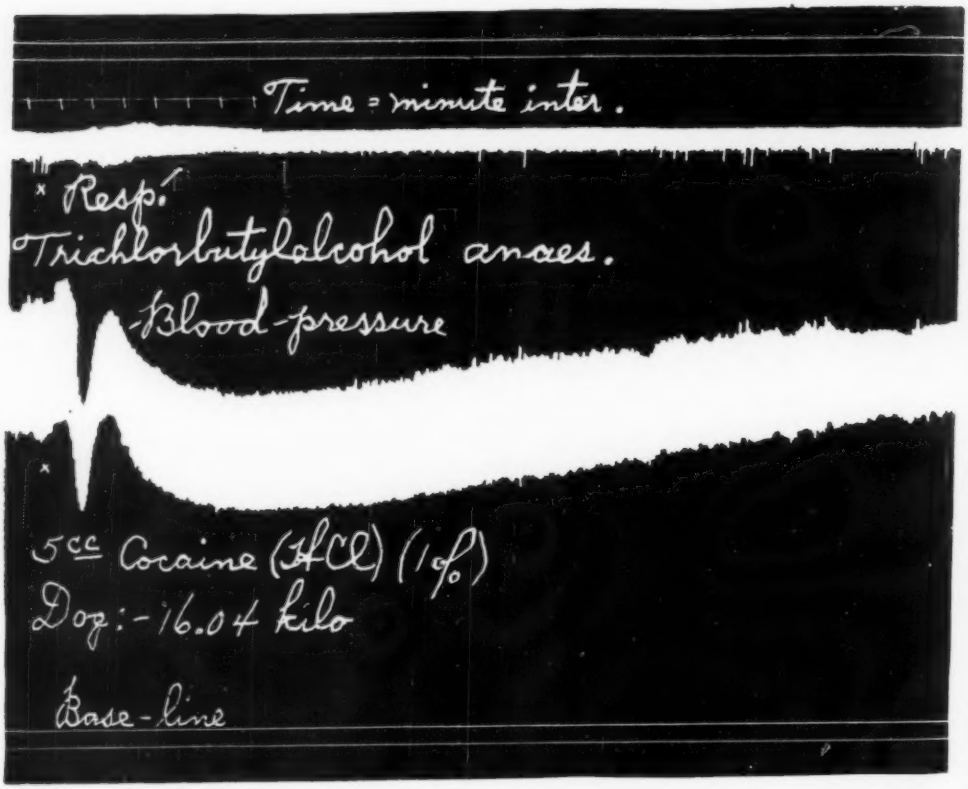

Fig. 4.

Sollmann suggests the following treatment of poisoning by local anesthetics: "If the drug has been taken by mouth, evacuation and chemic antidotes are indicated. If the anesthetic was injected, absorption should be checked by ligation, epinephrin or cooling. If symptoms have developed the head should be lowered; collapse treated by aromatic ammonia and caffeine; convulsions allayed by ether or chloral; and artificial respiration applied as needed; amyl nitrite has been recommended. Epinephrin and ouabain, intravenously, and cardiac massage are useful in animals (Eggleston and Hatcher, 1919), but have not been tried sufficiently in the clinic. Whatever delays death, is likely to save the life, in view of the rapid destruction of

these products. As prophylaxis the patient should be recumbent, if the operation permits. With nervous patients, it is advisable to inject morphine 15 minutes before the local anesthetic, and to delay the start of the operation till 20 minutes after the injection of the local anesthetic (Mayer et al, 1920)"

We have attempted to find a remedy which would not only prevent undesirable reactions from therapeutic and sublethal doses, but which would also eliminate convulsions and prevent death from fatal doses. The results of our work are reported in the following:

In the majority of experiments we used butyn as the local anesthetic. It was injected subcutaneously into rabbits and dogs in doses above fatal as well as in sublethal doses, sufficient to produce convulsions.

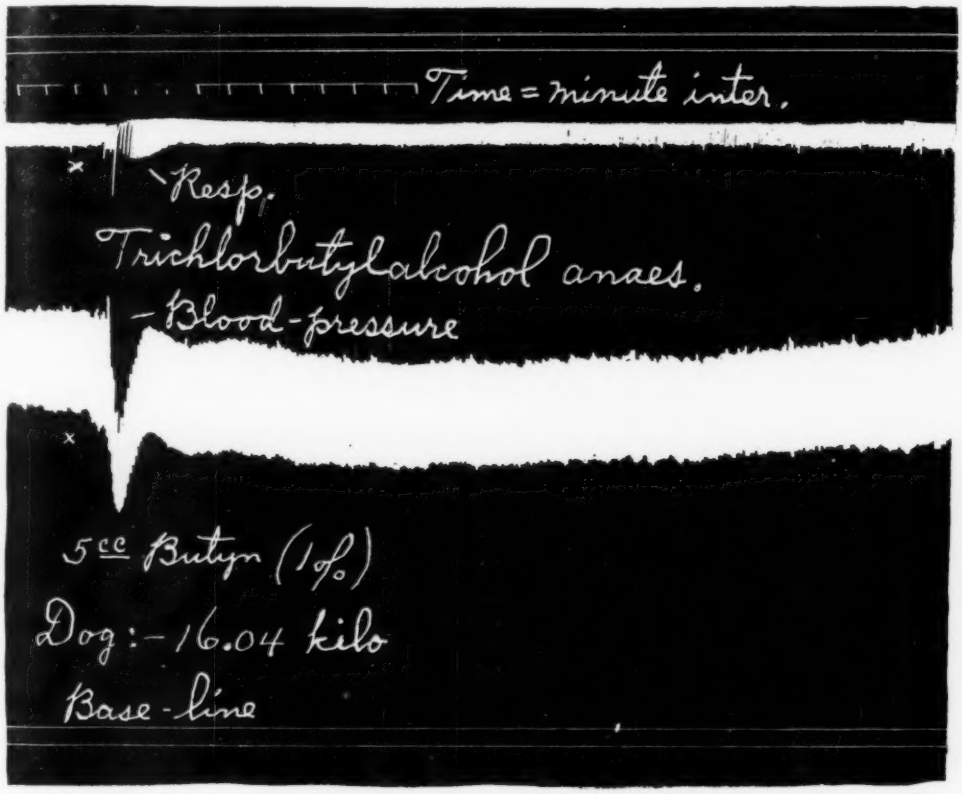

Fig. 5.

The following drugs were investigated for their detoxicating action:

Epinephrin
Caffeine Citrate
Hyoscine Hydrobromide
Morphine Sulphate
Atropine Sulphate
Hydrastinine
Pituitary Solution.

The use of pituitary solution was suggested by J. A. Higgins, and since it appeared to be more promising than the others it alone was subjected to further experiments, including its use in cocaine poisoning also.

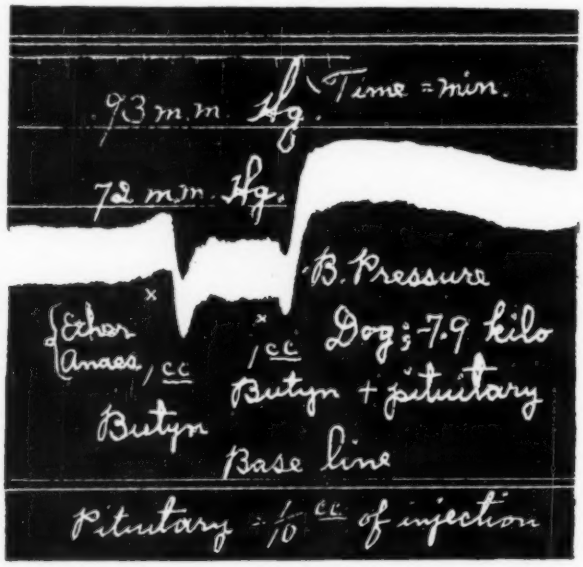

Fig. 6.

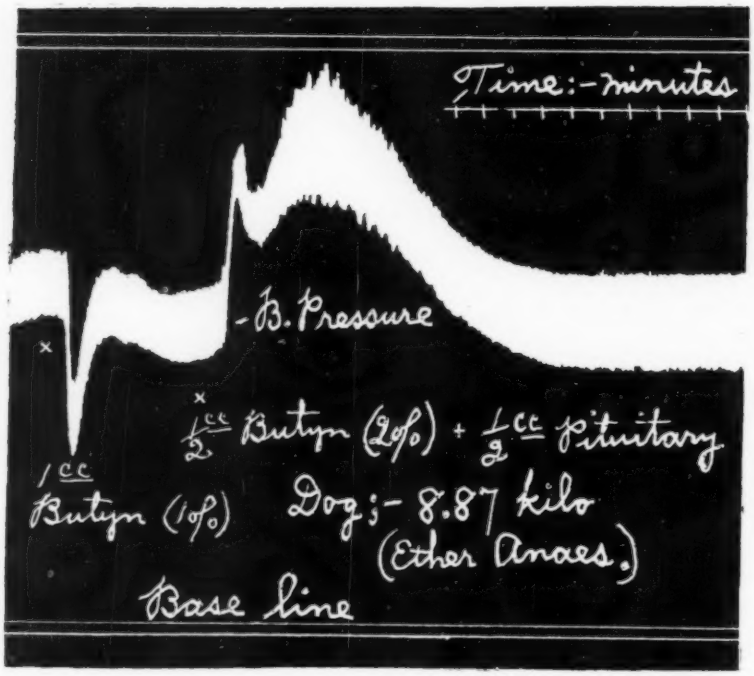

Fig. 7.

The pituitary solution we used was taken from samples sent over from our manufacturing department and found to be of the accepted standard activity on the isolated guinea pig uterus, and to produce the required sustained blood pressure rise in the dog.

The minimum fatal dose of butyn subcutaneously in rabbits and dogs is 55 milligrams per kilogram bodyweight (Tables II and IV). The minimum fatal dose of cocaine subcutaneously in dogs is 45 milligrams per kilogram bodyweight (Table IV).

Rabbit No. 2, weight 1.67 kg.; dose 55 mg. of butyn per kg. bodywt.; total dose 0.09185 grams. Total amount of a 5% solution 1.82 c.c. injected subcutaneously at 2:02 P. M. convulsions set in at 2:11 P. M. At the onset of convulsions, 3/4 of a c.c. of pituitary solution was injected intravenously. This injection immediately stopped the convulsions and the rabbit survived the fatal butyn injection.

Rabbit No. 3, weight 1.77 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1062 grams. Total amount of a 5% solution 2.13 c.c. injected subcutaneously at 11:25 A. M. Onset of convulsions at 11:28 A. M. Injected 1 c.c. of pituitary solution at 11:30 A. M. This injection checked the convulsions in a few seconds and the rabbit survived the otherwise fatal butyn injection.

Rabbit No. 4, weight 2 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.12 grams. Total amount of a 5% solution 2.4 c.c. injected subcutaneously at 1:41 P. M. Onset of convulsions at 1:49 P. M. Injected 1 c.c. of epinephrin solution 1:10,000 intravenously 1 minute after the onset of convulsions. The animal remained quiet for a short period after this injection, but convulsions returned and the rabbit died at 2:38 P. M. Postmortem examination revealed a straw colored serum flowing from the nostrils, a pinkish tinge around the mouth, congestion of the liver, heart veins distended; the lungs contained a small amount of serum, but there was no indication of a rupture of the lung vessels.

Rabbit No. 5, weight 2.05 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.123 grams. Immediately before injecting the butyn, 1 c.c. of a 1% caffeine citrate solution was given subcutaneously (at 1:02 P. M.). At 1:04 P. M. 2.46 c.c. of a 5% butyn solution was injected subcutaneously. The caffeine injection did not stop the convulsions and the rabbit died at 2:04 P. M.

Rabbit No. 6, weight 2.57 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1542 grams. At 1:55 P. M. a subcutaneous injection of 1/2 c.c. of pituitary solution was given, and immediately following this injection, 3.08 c.c. of a 5% butyn solution was administered subcutaneously. (1:57 P. M.) The rabbit lies down quietly. No convulsions occurred and the animal was apparently well recovered at 3 P. M.

Rabbit No. 7, weight 1.89 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1137 grams; total amount of a 5% butyn solution in 2.27 c.c. injected subcutaneously at 2:38 P. M. Convulsions began at 2:46 P. M., and an injection of 1 c.c. of 1% caffeine citrate solution was then administered intravenously. This injection did not stop the convulsions; the animal was still on its side at 4:50 P. M.; it recovered slowly.

Rabbit No. 8, weight 1.7 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.102 grams. In this rabbit the caffeine solution was administered immediately before the butyn (same as in rabbit No. 5). 1 c.c. of a 1% caffeine citrate solution was injected subcutaneously at 3:30 P. M. followed by a subcutaneous injection of 2.04 c.c. of a 5% butyn solution at 3:32 P. M. The caffeine did not prevent convulsions, but the animal survived the fatal dose of butyn and was apparently normal the following morning.

Rabbit No. 9, weight 2.02 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1212 grams. In this animal the caffeine dose was doubled. One c.c. of a 2% caffeine citrate solution was injected subcutaneously at 10:27 A. M., followed by a subcutaneous injection

of 2.42 c.c. of a 5% butyn solution at 10:28 A. M. Convulsions set in at 10:35 A. M., and the animal died at 10:41 A. M.

Rabbit No. 12, weight 1.35 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.081 grams. Immediately before injecting the butyn, the animal received 1/100 of a grain of hyoscine hydrobromide subcutaneously. Total amount of a 5% butyn solution injected subcutaneously 1.62 c.c. The animal had convulsions of a mild type, and recovered (3:45 P. M.) from the fatal dose of butyn.

Rabbit No. 13, weight 1.71 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1026 grams. Immediately before giving the Butyn injection the animal was given 1/4 of a grain of morphine sulphate subcutaneously. Total amount of a 5% butyn solution injected 2.05 c.c. The morphine did not prevent the convulsions and the animal died 14 minutes after the butyn injection.

Rabbit No. 14, weight 1.73 kg.; dose 60 mg. of butyn per kg. bodywt.; total 0.1178 grams. The animal was first given 1/100 of a grain of atropine sulphate subcutaneously, followed immediately by 2.36 c.c. of a 5% butyn solution subcutaneously. Some convulsive movements of a mild type; the animal tries to get up 47 minutes after the butyn injection. Recovered.

Rabbit No. 16 weight 1.745 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1047 grams. The animal received first a subcutaneous injection of 1/64 grains of Hydrastinine followed immediately by 2.09 c.c. of a 5% butyn solution. The Hydrastinine did not check the convulsions but the animal survived the fatal butyn dose.

Rabbit No. 17, weight 1.575 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.0945. Immediately before giving the butyn injection, the animal received 1 c.c. of a 1:10,000 solution of epinephrin subcutaneously. Total amount of a 5% butyn solution injected 1.89 c.c. The epinephrin did not prevent the convulsions, but the animal survived the fatal butyn dose.

Rabbit No. 21, weight 2.17 kgs.; dose 60 mgs. per kg. bodywt.; total dose 0.1302 grams; total amount of a 15% solution of butyn 0.87 c.c. injected subcutaneously at 11:31 A. M. No convulsions occurred up to noon, probably due to faulty technic in the injection. At 12:02 P. M. 0.75 c.c. of a 15% solution was injected subcutaneously and at 12:11 P. M. convulsions set in. At this time 1 c.c. of a 1:1000 epinephrin solution was injected subcutaneously. The epinephrin did not prevent the convulsions and the rabbit died at 12:21.

Rabbit No. 18, weight 1.85 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.111 grams. The animal was given a subcutaneous injection of 2.22 c.c of a 5% butyn solution at 4:35 P. M. At 4:44 P. M. convulsions started and at this time 1/100 of a grain of hyoscine hydrobromide was injected subcutaneously. This injection did not prevent the convulsions and the animal died at 4:52 P. M.

Rabbit No. 19, weight 2.65 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.159 grams. Total amount of an 8% butyn solution, 2 c.c. injected subcutaneously. At the onset of convulsions, 1 c.c. of pituitary solution was injected intravenously. The convulsions immediately ceased, the animal becoming completely relaxed and "flexible." Recovered.

It will be seen from these results that the detoxicating action of pituitary solution was far superior to that of the other drugs. When pituitary solution was given subcutaneously before a fatal dose of butyn was injected, there were no convulsions and the animals recovered. Administering the pituitary solution intravenously after the onset of convulsions from a fatal dose of butyn, it immediately stopped the convulsions and saved the animal from death. The results from these intravenous injections of pituitary solution were so striking and so unfailing, that a series of experiments of this

character were demonstrated by one of us (J. A. H.) at the meeting of the National Anesthesia Research Society held in Cincinnati during the week of January 8, 1923. In order to find out whether or not pituitary solution injected subcutaneously after the onset of convulsions from a fatal dose of butyn would be of benefit also, the following experiment was made:

Rabbit No. 20, weight 3.34 kg.; dose 60 mg. per kg. bodywt.; total dose 0.2004 grams. Total amount of a 15% solution of butyn 1.34 c.c. injected subcutaneously at 11:05 A. M. Onset of convulsions at 11:13 A. M. at which time 1 c.c. of pituitary solution was injected subcutaneously. This injection did not stop the convulsions and the animal died at 11:21 A. M.

These experiments demonstrated the desirability of injecting pituitary solution simultaneously with the butyn. A series of dogs were therefore given subcutaneous injections of butyn in doses above the fatal and containing various amounts of pituitary solution. It was found that a relatively small dose of pituitary was sufficient to prevent convulsions and death. Still smaller doses of pituitary solution were sufficient to eliminate the symptoms ordinarily produced by high sublethal doses of butyn.

Dog No. 32, weight 9 kg.; dose 60 mg. of butyn per kg. bodywt.; total 0.54 grams. Total amount of a 15% butyn solution, 3.6 c.c., containing 0.09 c.c. of pituitary solution, injected at 11:17 A. M. No convulsions occurred. There was some incoordination and some excitement. Animal recovered.

Dog No. 33, weight 8.73 kg.; dose 30 mg. of butyn per kg. bodywt.; (high sublethal dose); total dose 0.2619 grams; total amount of 15% butyn solution, 1.75 c.c., containing 0.022 c.c. (approximately 1/45 c.c.) of pituitary solution injected subcutaneously at 1:55 P. M. Bowel movements and vomiting at 2:07 P. M. Animal somewhat excited. Vomits again at 2:30 P. M. Bowel movements at 2:35 P. M. Dog somewhat nervous. No convulsions. Recovery at 3:30 P. M.

Dog No. 34, weight 4.48 kg.; dose 40 mg. of butyn per kg. bodywt. (high sublethal dose); total dose 0.1792 grams. Under ordinary circumstances this dose produces violent convulsions. Total amount of a 15% butyn solution, 1.194 c.c., containing approximately 1/100 of a c.c. of pituitary solution per kg. bodywt. (Total dose of pituitary solution 0.05 c.c.) injected subcutaneously at 10:30 A. M. No convulsions; animal is perfectly quiet; no excitement. Recovery at 12:15. The animal did not refuse food offered at this time (in contrast to animals who have received high sublethal doses of cocaine, who always refuse food for a long period of time after apparent recovery.)

In view of these excellent results from the use of pituitary solution in treating poisoning by butyn, a few experiments were conducted to show whether it would be beneficial also in cocaine poisoning; if so, it would seem highly probable that pituitary solution would be of equal benefit in the treatment of poisoning by all the anesthetics of this group.

Dog No. 35, weight 5.02 kg.; dose 25 mg. of cocaine per kg. bodywt. This is a high sublethal dose which always produces great excitement, incoordination, extreme anxiety, followed by very violent convulsions. Total dose 0.1255 grams. Total amount of 15% cocaine solution, 0.84 c.c., containing approximately 1/100 of a c.c. of pituitary solution per kg. bodywt. of animal (total dose of pituitary solution 0.050 c.c.). Injection made at 10:50 A. M. Incoordination a few minutes after the injection; the animal was somewhat excited, but no convulsions occurred. Animal apparently recovered at noon, but refuses food.

Dog No. 36, weight 4.82 kg.; dose 50 mg. of cocaine per kg. bodywt. This dose is above fatal. (M. F. D. subcutaneously in dogs 45 mg. per kg. bodywt.) Total dose 0.241 grams. Total amount of a 15% cocaine solution 1.61 c.c., containing approximately 1/100 of a c.c. of pituitary solution per kg. bodywt. of animal. Total dose of pituitary solution 0.05 c.c. A few minutes after the injection there was an increase in saliva; the animal became extremely excited; pupils very dilated. Animal turning its head unceasingly from one side to the other in extreme anxiety; marked incoordination; muscular weakness of hind quarters; animal fell over on one side and was unable to arise. At 12:30 the animal attempted to get up, but was too weak in the hind limbs. Later it finally succeeded in getting up and at 1:30 P. M. the animal moved about in an excited condition; the flow of saliva decreased; the animal refused food; there were some twitching movements of the head, but no convulsions. Animal recovered.

The action on the blood pressure of butyn solution and pituitary solution mixed compared to that of butyn solution alone, is shown in Figs. 6 and 7. In Fig. 6 the dog was given an intravenous injection of 1 c.c. of a 1 per cent butyn solution, producing as usual an abrupt fall in blood pressure. Five minutes later another 1 c.c. of a 1 per cent butyn solution, containing $\frac{1}{10}$ of a c.c. of pituitary solution was given; this injection caused only a very slight fall of blood pressure followed by an immediate high and sustained rise.

In Fig. 7 a dog received 1 c.c. of a 1 per cent butyn solution intravenously, producing an immediate abrupt fall in blood pressure; a few minutes later an injection of $\frac{1}{2}$ c.c. of a 2 per cent butyn solution mixed with $\frac{1}{2}$ c.c. of pituitary solution was injected intravenously and this injection caused an immediate and high rise of blood pressure. The blood pressure did not fall below normal thereafter.

CONCLUSIONS

The safety of a local anesthetic in human therapy should not be judged from its toxicity compared to cocaine on experimental animals.

In determining the toxicity of a local anesthetic on animals, as many different species as possible should be investigated, preferably higher experimental animals. The toxicity should not be measured by comparison with cocaine as a standard without taking into account the fact, that cocaine is highly selective in toxicity, that is, the higher the development of the brain in the animal, the more toxic is the cocaine. The comparative variation in toxicity depending upon the species of animal, mode of application, concentration of solution, rate of injection, rate of absorption, rate of destruction in an excretion from the body, as well as the comparative action on blood pressure and respiration, should be determined.

With these pharmacologic data in mind, final judgment on the safety and the field of usefulness of a promising local anesthetic in human practice, should be passed only after sufficient clinical experience.

Various drugs were investigated for their detoxicating action in the treatment of poisoning by the local anesthetics of the so-called "cocaine group" (alkamine esters of aromatic acids). Pituitary solution appeared to be superior to the other drugs investigated, and holds out promise both as a preventive of undesirable symptoms from sublethal doses as well as an anti-

dote in poisoning by fatal doses of these local anesthetics. Judging from our experiments, small therapeutic subcutaneous doses of pituitary solution injected simultaneously with the local anesthetic are sufficient to eliminate the fall in blood pressure from therapeutic doses as well as the convulsions from higher doses, and death from fatal doses. Used as an antidote in cases where the convulsions have already set in, pituitary was effective only when administered intravenously. It deserves clinical trial. It should, of course, not be used in pregnant or in high blood pressure patients.

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ATRESIA OF THE PULMONARY ARTERY IN A CONGENITALLY DEFECTIVE HEART*

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CONGENITAL heart disease, a condition first discussed by Senac in 1749, involves the existence of anomalies in the anatomical structure of the heart or great vessels, of intrauterine origin, leading to disturbances in the circulation. Every case, especially if it is one of the rarer varieties, should be recorded, since the congenitally defective or malformed heart is an object of widespread interest to the anatomist and embryologist, as well as to the pathologist and clinician. Recently an example of the rare congenital cardiac condition, pulmonary atresia, was observed at necropsy in a four and one-half months old female infant. The following defects were present: Patency of foramen ovale, ventricular septum, and ductus arteriosus; atresia of pulmonary artery and valve and conus pulmonalis; origin of aorta from right ventricle; complete cleft palate and right hare lip and mobile cecum.

CASE HISTORY

Case 1 (A395079). The child, aged two and one-half months, was admitted to the Worrell Hospital June 16, 1922, for plastic operation for harelip on the right and complete cleft palate. Her father, a physician, said that she was the third of three children, the other two being well formed, and healthy. The child's birth had been uneventful. She weighed seven pounds at birth, but had gained very little since; for the first three weeks she was very cyanotic and continued to have spells of strangulation and cyanosis. Her weight at the time of examination was about 8 pounds, she was emaciated and the skin was loose and flabby. The left upper eyelid was shortened, causing extreme difficulty in opening the eye; the left eyeball was about 60 per cent normal in size, but was perfect in function and by objective examination. There was some nystagmus at times on looking to the left. The sagittal suture extended quite far down on the forehead. The veins of the upper abdomen were distended. Occasionally the child breathed slowly, at which time a continuous hum was heard over the base of the heart and in the pulmonary area, rapidly diminishing toward the left and apex. The manubrium sterni was somewhat prominent. Four days after the first examination the color was better. The erythrocytes numbered 4,880,000; the leukocytes 6,900, and hemoglobin was 80 per cent. The urine was normal.

August 9, 1922, the patient was operated on for cleft palate. There was marked cyanosis and straining before ether was taken, but she did well under it and the postoperative recovery was uneventful. August 16, 1922, the operation for harelip was performed. The temperature rose to 103 after operation and remained there. There was considerable cyanosis, and death occurred suddenly about fifteen hours after the operation.

At necropsy, findings as follows were noted: The body was poorly developed and poorly nourished, 56.5 cm. in length. The left eye was smaller than the right; the iris of the left eye was 7 mm. in diameter; of the right eye, 10 mm.; the pupil of the

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Received for publication, November 8, 1922.

left eye, 1 mm.; of the right eye, 1.5 mm. There was considerable cyanosis about the face, lips, and neck. The cecum and ascending colon were abnormally mobile, and were located just to the right of the middle line. The ductus venosus was closed at its portal and hepatic ends. The middle and lower lobes of the right lung were slightly collapsed, and somewhat flabby in consistency; a small cut section readily sank in water, although the entire mass of lung tissue floated, together with the heart. Otherwise both lungs appeared to be normal.

The right auricle was enormously dilated. The superior vena cava and all the coronary vessels were distended with blood. The cardiac muscle was bright red and firm. The wall of the right ventricle was markedly thickened, averaging 0.5 cm.; the papillary muscles were correspondingly large and prominent (Fig. 1). The depth of the right ventricle was 2.5 cm. In the upper part of the interventricular septum there was an oblique oval aperture, 11 by 3 mm. All that remained of the conus arteriosus and the pulmonary valves was a minute depression 4 mm. in length and 1 mm. in

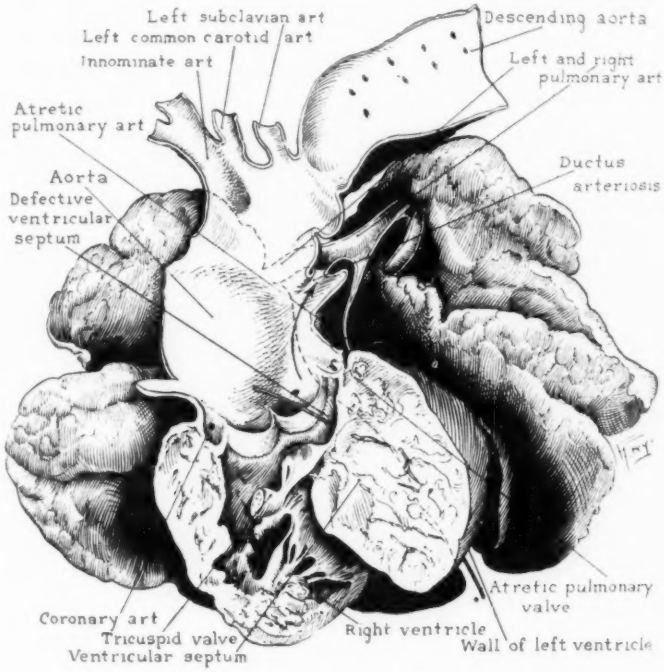

Fig. 1.—Posterior view of heart and lungs.

width, in the wall of the right ventricle, to the right and at the base of the interventricular septum, beside the opening in the septum, and on a level with the aortic cusps. In this shallow depression could be made out six oblique, minute subdepressions, as well as two above them. The whole mass was surrounded by a smooth, muscular, elliptical "plateau." About 4 mm. above this a narrow fibrous cord 0.5 mm. in diameter, entered into the muscle at the top of the ventricular septum and ended blindly, being lost in the muscle mass. This cord was barely dissectable grossly, had a minute lumen which passed over in the fibrous cord for 8 mm. to expand and communicate definitely with the right pulmonary artery, at its point of juncture with the latter, the circumference of the channel being 4 mm. The right and left pulmonary arteries presented normal lumina of 10 mm. circumference. The left pulmonary artery, near its point of juncture with the right, was connected with the arch of the aorta, opposite the arc from which the head vessels were given off, by a duct 5 mm. in length and 8 mm. in circumference, with slightly roughened intimal lining, the patent ductus

arteriosus. The aorta opened directly out of the right ventricle, and measured 36 mm. in circumference at its root. Three delicate, competent aortic valves were present, together with normal openings of the right and left coronary arteries. The aorta maintained its large circumference until the head vessels and ductus were reached, beyond which it presented a definite stenosis, being reduced to 15 mm. in circumference. One cm. beyond, it again widened to a circumference of 30 mm. The head vessels passed off normally. Two mm. below the origin of the left subclavian a minute accessory vessel originates. The paired intercostal arteries arise normally. The aorta at its point of stenosis presented a somewhat roughened intima, with a slightly elevated transverse ridge distally. Another "V"-shaped elevation occurred at the point of separation of the ductus and the narrowed aorta; and a transverse ridge stretched across the left pulmonary artery directly opposite the point of entry of the ductus.

The foramen ovale was patent (Fig. 1), presenting a complete opening, 10 by 3 mm. The openings of the coronary sinus, the superior and inferior venae cavae were in their normal positions. The wall of the right auricle and the auricular appendage were excessively hypertrophied. The tricuspid value measured 4.3 cm. in circumference and presented a delicate line of closure. The pulmonary veins opened normally into the left auricle. The mitral valve was delicate and competent, and measured 3.1 cm. in circumference. One of its cusps was continuous through the defective ventricular septum with one of those of the tricuspid valve. The depth of the left ventricle was 2.3 cm.; the average thickness of its wall was 0.5 cm., small as compared with that of the right ventricle.

DISCUSSION OF THE LITERATURE

In 1880 Stifel collected forty-six cases of pulmonary atresia from the literature. In 1898 Vierordt added twenty-four cases, bringing the total to seventy, although there may be some overlapping of the two series. The nine cases reported in the literature since 1898 bring the total number of cases of pulmonary atresia to seventy-nine. Vierordt estimated the minimum number of reported cases of pulmonary stenosis to be three hundred.

The first paper on congenital atresia of the pulmonary artery was written by Hunter in 1783. There followed at infrequent intervals reports of isolated cases, until in 1858 Peacock published his classic on cardiac malformations. Among one hundred eighty-one instances of congenital heart disease which he assembled, one hundred nineteen (66 per cent) presented pulmonary stenosis or atresia (ninety, stenosis, twenty-nine, atresia). Rauchfuss' series of 1878 included eighty-one of stenosis and thirty-three of atresia; Vierordt's series, eighty-three of stenosis and twenty-four of atresia. In 1891 von Etlinger collected two hundred sixty-six cases of stenosis and atresia.

Stifel made a thorough study of pulmonary atresia in 1880. He found forty-six cases recorded to date, in six of which the pulmonary artery was represented by a solid, fibrous cord; in thirty, the pulmonary orifice was completely closed, and in ten, the obliteration was indefinite. Thirty-five were accompanied by a defective ventricular septum, thirty-eight by a patent ductus arteriosus, and twenty-eight by a patent foramen ovale. Eleven cases of the series showed dextroversion. Fifty per cent of the patients died within the first three months, 74 per cent within the first year, 90 per cent within the first twelve years, while one survived to the age of thirty-seven. Hirschfelder states that 78 per cent of the patients with atresia die during the first year, while only 9.7 per cent of those with stenosis die during the same period,

although 64 per cent die before puberty. Rauchfuss places the average duration of life in pulmonary stenosis at nine years, and in pulmonary atresia at three years.

Maude Abbott has analyzed six hundred thirty-one cases of congenital heart disease, with postmortem reports all from reliable sources, thus presenting a fairly accurate basis for a statistical study. The series includes ninety cases of pulmonary stenosis and twenty-nine of pulmonary atresia. Of the latter the ventricular septum was defective in 72 per cent; the ductus arteriosus and the foramen ovale were patent in 65 per cent.

Eleven of twenty-four of these atresia cases had defective ventricular septum and patent foramen ovale, the same combination of defects being present in the case reported here. The ages of patients ranged from thirteen days to thirteen years. Five were males, two were females. Seven had patent ductus arteriosus; seven had dilated aortas; nine, dextroversion of the aorta; seven, hypertrophy of the right auricle; eight, hypertrophy of the right ventricle; and two, hypertrophy of the left ventricle. In six cases there was marked cyanosis; in two, moderate; and in one, slight. In four, there were delayed development, systolic murmur, and dyspneic attacks; in three, clubbing of fingers; in two, thrill, pulsation, and dyspnea; and in one, precordial bulge and increased second pulmonic sound. Hence in the entire series of cases collected (to 1908) there are but seven which resembled the case I report here, that is, with defective ventricular septum, patent foramen ovale and ductus arteriosus, and atresia of the pulmonary artery. Of the twenty-one cases with defective ventricular septum, the aorta arose from both ventricles above the opening in seven, and entirely from the right ventricle in fourteen.

In the same article Abbott describes a specimen from the McGill Pathological Museum, presented by Sir William Osler, which closely resembles the one I have described.

In recent years occasional cases of pulmonary atresia have been recorded in the literature, such as those by Grothe in 1898, Curl in 1905, McCrae 1906, and Burnier in 1907. In 1915 Stoddard collected four cases that had been reported by D'Espine in 1908, Bach in 1909, Vallois in 1910, and by Edwards in 1911. Edwards emphasizes the fact that in complete pulmonary stenosis, or atresia, the blood reaches the lungs by one of five routes: (1) patent foramen ovale, (2) defective ventricular septum, from the right into the left side of the heart, and by way of the aorta, (3) through the patent ductus arteriosus, (4) the bronchial arteries, or (5) the anomalous branch of the left subclavian artery (one instance). Carpenter describes an aorta arising from both ventricles (four-fifths from the left), a small pulmonary artery (0.31 cm. in diameter) with two semilunar valves, a patent ductus arteriosus, the semilunar valve efficiently guarding the foramen ovale, but displaying a tiny perforation, in a ten weeks' child, without cyanosis. Bach's case of seven weeks showed cyanosis, and the pulmonary artery arising abruptly from the heart muscle, the presence of stenosis and insufficiency of tricuspid

valve, persistent foramen ovale and ductus arteriosus. The walls of the right ventricle were very thick and the cavity was rudimentary.

D'Espine reports a case of "cyanose congénitale paroxystique," the fifth on record, a condition first described by Variot, as an intermittent congenital cyanosis with periods of freedom, or near-freedom, and caused by "hypertrophy of the right ventricle; communication of aorta with right ventricle; stenosis of conus pulmonalis; and an interventricular communication." In Burnier's case the pulmonary artery terminated blindly in the heart. D'Espine notes that the pulmonary artery and valves are present, but very small. He believes the predisposing cause of the cyanosis to be the mixture in the aorta of the two blood streams which have come from the two ventricles. The right ventricular hypertrophy in these cases causes an increase in the proportion of dark venous blood in the aorta, and so brings about a latent predisposition toward cyanosis. Stasis of blood brought about suddenly by an acute dilatation of the right ventricle due to disease, muscular fatigue, emotion, digestive influences, and so forth, can easily disturb the delicate equilibrium and convert latent into active cyanosis, since insufficient blood then passes up the aorta, through the ductus, and into the pulmonary circulation. During these paroxysms of cyanosis there are often present edema of the eyelids or extremities, great increase of percussion dullness to the right, and precordial heave.

From an analysis of 242 necropsies in cases of congenital heart disease, Holt and Howland (1922) find that a defective ventricular septum is the most common lesion, occurring in 149 cases, although it is the sole lesion in only five of these. It is most common in association with pulmonary stenosis or atresia, namely in ninety-two cases.

GENERAL DISCUSSION

Pulmonary stenosis and pulmonary atresia must be differentiated sharply, although they represent merely different degrees of the same pathologic process. Stenosis is a narrowing without obliteration; atresia is a narrowing, with obliteration, of the functional channel. Pulmonary stenosis is the most common variety of congenital heart lesion, and is from three to eight times as common as atresia.

As to the cause of atresia of the pulmonary artery, two possibilities present themselves: Endocarditis in fetal life is applicable in such a limited number of instances that practically it may be dismissed. Osler could not imagine "a fetal endocarditis localized to so small an area as the pulmonary valves must be before the eighth week of fetal life." The second possibility, defective development of the pulmonary artery, has much to support it. At about the eighth week of fetal life the pulmonary artery becomes separated from the truncus arteriosus together with the sixth branchial arch on the left side, the communication with the descending aorta persisting as the ductus arteriosus. The truncus pulmonalis, because of the twisting of the cardiac tube, protrudes ventrally, while the aortic portion lies dorsally. The truncus pulmonalis thus represents the ventral half of the truncus arteriosus

and springs directly from the infundibulum of the right ventricle. Stenosis or atresia may occur as a result of retarded development in the infundibulum below the valves, at the valves, or in the pulmonary artery itself. Atresia is caused by unequal division of the truncus arteriosus by the septum of the bulb, which normally presents its convexity downward toward the pulmonary half of the truncus, approaches the truncus, and fuses with it as far down as its mouth. The cause of the unequal division is probably imperfect development of the fifth branchial arch.

His claims that such disturbances of developmental conditions are brought about by "insufficient nourishment, insufficient aeration of blood, and mechanical causes resulting from malpositions of uterus, disturbed placental circulation, and so forth." Panum has shown them to be producible experimentally in birds by raising the temperature of incubation, or by inducing fever in the mother. Keith, as a result of a series of personal observations on conus stenosis and the study of two hundred seventy malformed hearts in the museums of London, has advanced the view that cardiac defects are nearly always developmental in origin, and that pulmonary stenosis is usually due to subinvolution of the bulbis cordis, the development being arrested at a stage when this fourth primitive chamber existed in the heart.

The course of the circulation of the blood in the heart in the case here reported must be as follows: From the right ventricle into the aorta, through the ductus arteriosus into the pulmonary arteries, back through the pulmonary veins into the left auricle and ventricle, through the defective interventricular and interauricular septums into the right ventricle and auricle and also from systemic veins into the right auricle and ventricle. This explains the great bulk of work thrown on the right side of the heart, and its subsequent hypertrophy and dilatation. The left side is practically useless except as a passageway.

Why is the aorta given off from the right ventricle? Because in these cases of atresia, practically all of which show this phenomenon of dextroversion (Rechtslage), the deflection of the ventricular septum to the left is so great that as a result the aorta lies in the axis of the right ventricle.

The symptoms which may be expected in such cases are briefly: cyanosis, also known as the morbus coeruleus or blue sickness, shown at birth in seventy-four of Peacock's one hundred one cases and in practically all within one or two years; dyspnea, especially on exertion, headaches, cold hands and feet, and a tendency to bleed easily. During the attacks of strangulation the patient becomes blue or black, and a fainting spell or epileptiform convulsion may result from the venous stasis and cerebral ischemia. There is a precordial bulge, a wavy systolic impulse, increased percussion dullness on both sides, decreased or absent second pulmonic sound, a rough systolic thrill in the pulmonic area together with a murmur, transmitted up and to the left, but not into the vessels of the neck. The liver and spleen may be large. A polycythemia of six to nine billion is the rule, with a hemoglobin of 110 to 130 per cent. Secondary lesions often exist, such as distention of the arteries and veins, and malformation of arterioles and venules. The former results

in stasis, edema, frequent hemorrhages, and retarded growth in stature and intelligence; the latter produces terminal capillary sinuses in the skin viscera, and retina (diagnosed by ophthalmoscopic examination). Clubbing of the fingers is often present and is occasioned by excessive connective tissue proliferation as a result of the venous stasis.

The case presented seems to be unique in the combination of associated cardiac anomalies displayed, and remarkable in the comparative normality of the child aside from the congenital hare lip and cleft palate. The cyanosis was of such a remittent type, associated with periods of comparative freedom, as to justify placing this case in D'Espine's group of "cyanose congénitale paroxystique." Although the exact number of cases of pulmonary atresia recorded to date cannot be determined accurately, this may be called the eightieth.

SUMMARY

A case of congenital atresia of the pulmonary artery, pulmonary valve, and infundibulum of the right ventricle is described in conjunction with other developmental anomalies: origin of the aorta from the right ventricle; presence of a patent ductus arteriosus, and defective ventricular and auricular septums; complete cleft palate and right hare lip. The ascending aorta, as far as the opening of the ductus arteriosus, thus served as both a pulmonary artery and an aorta, the ductus arteriosus conveying blood from the aorta to the lungs through the widely patent right and left branches of the atretic pulmonary artery. The child lived four and one-half months in fairly good health, and died only after a plastic operation. Prior to operation she had shown intermittent cyanosis and attacks of strangulation.

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THE INCREASE IN VIRULENCY OF NONPATHOGENIC MICRO-ORGANISM BY CHEMICAL SUBSTANCES*

By R. H. Lee, B.S., and Lloyd Arnold, M.D., Chicago, Ill.

HANS MUCH[4] records some observations made with nonpathogenic bacteria that were apparently changed into a pathogenic type by simultaneously injecting in the experimental animal a weak solution of lactic acid.

It has been known for some time that lactic acid increases the virulence of some of the pathogenic microorganisms. This has been explained more upon the basis of an impairment of the defensive mechanism of the host than upon an actual change in virulency of the invading bacteria. Gabritschevsky[2] found lactic acid to be indifferent to or possibly exerting a "negative chemotaxis" on leucocytes.

In the experiments reported by Much there was an actual change from a typical nonpathogenic or avirulent to a pathogenic or virulent type. Bac. mycoides, B. proteus and even B. subtilis caused acute infections with early death, and the respective organism could be isolated from the blood and organs after death. The strains Much used were isolated from the air and contaminated medium in his laboratory. He used mice, guinea pigs and rats. The lactic acid was in 0.01 to 1.0 per cent concentrations. For mice 0.2 c.c. of the dilute lactic acid was used. This was injected at the same time as the nonpathogenic microorganism, either in the same place or in a different place. If dead bacteria were used no change was produced. His experiments with B. subtilis injections combined with lactic acid were negative, but when lactic acid was added to bouillon, to make a concentration of 1.0 to 0.001 per cent lactic acid in bouillon and B. subtilis was grown in this lactic acid bouillon, it became very virulent for mice. Much claimed this artificially produced virulence was caused by the action of the lactic acid on the living microorganism.

Bachmann[1] repeated the experiments recorded by Much, but found that the lactic acid injection had no effect at all on the microorganisms. This author used Proteus vulgaris, Proteus X19, B. subtilis, B. typhosus, B. shiga and kruse. The experimental animals used were mice and guinea pigs. The results were all negative. Bachmann thinks probably "dass die vom Much erzielten Ergebnisse an besondere biologische Eigenschaften seiner Kulturen geknuepft sind, die erst unter dem Einfluss des Milchsaur-zusetzes in die Erscheinung treten." Lange and Yoshioka[3] repeated the experiments of Much with negative results.

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Received for publication, Nov. 15, 1922.

TECHNIC

Plain Liebig peptone (Fairchilds) bouillon was used as fluid medium. 2.5 per cent agar was added to make solid medium. B. subtilis was an old laboratory strain. B. proteus was obtained from Prof. Kendall.

In order to grow each of these microorganisms in a bouillon containing lactic acid, we determined the maximum amount of the lactic acid that could be added to the bouillon that would support growth of the respective bacteria. 0.17 per cent lactic acid in bouillon was the maximum concentration for B. subtilis; even with this amount, the third 24 hour transplantation showed a poor growth, it was then transferred to plain agar to rejuvenate the culture and after 24 hours' growth again transplanted to the lactic acid bouillon. The B. subtilis was kept going in this way for three weeks before the animal inoculations were started.

It was found that 0.08 per cent concentration of lactic acid in bouillon was all that would support growth of the B. proteus strain. It was transferred to plain agar every third day, as was done with the B. subtilis, to keep the growth of uniform density as compared with the plain bouillon control tube. This was also carried on for three weeks before animal inoculations were started.

The streptococci used were hemolytic and nonhemolytic strains isolated from the throats of patients suffering from diphtheria, also some strains from normal throats were used. No effort was made to grow the streptococci on lactic acid bouillon.

EXPERIMENTAL

B. subtilis.—One c.c. of a 24 hour culture of B. subtilis in 0.17 per cent lactic acid bouillon; 1 c.c. of a 24 hour culture of B. subtilis and 1 c.c. of 0.85 per cent lactic acid were injected subcutaneously in the back near the tail in two different mice. One c.c. of a 24 hour culture of B. subtilis in plain bouillon and 1 c.c. of 0.85 per cent lactic acid were injected separately in the same manner in two other mice; these served as controls. Four mice were used in this experiment. This was repeated six times, making a total of 24 mice in the whole series. All mice were alive after 30 days' observation.

B. proteus.—We used the same procedure with B. proteus as was used with B. subtilis. Six groups of 4 mice each were injected. One control B. proteus mouse died after 24 hours, this bacillus was recovered in pure culture from the heart's blood. The remaining 5 mice were living 30 days after injection. The lactic acid control mice were not affected. One of the 6 mice injected with B. proteus grown in lactic acid bouillon died three days after injection, B. proteus was recovered from the heart's blood. Three of the 6 mice injected with both B. proteus and lactic acid died, two 24 hours and one 3 days after injection. B. proteus was isolated from heart's blood in the one that died 3 days and in one of the two that died within 24 hours.

A 24 hour bouillon culture of B. proteus was killed by heating to 58° C. for 1 hour. One c.c. of this was injected subcutaneously in the same manner into each of six mice, into three of these 6 mice was also injected 1 c.c.

of a 0.85 per cent lactic acid. All of the mice injected with dead B. proteus lived. One of the mice injected with dead B. proteus and lactic acid died after two days, the other two were living after 30 days' observation.

Streptococci.—0.5 c.c. of a 24 hour bouillon growth of streptococci and 0.5 c.c. of a 0.85 per cent lactic acid were injected subcutaneously into mice. There were 16 strains of hemolytic and 9 strains of viridans used, all were nonpathogenic for mice. All the animals remained alive after a three weeks' period of observation. Twenty-two mice had been injected with streptococci, in a previous experiment to test their pathogenicity, and were alive 8 to 14 days after receiving the inoculation, were injected subcutaneously with 0.5 c.c. of an 0.85 per cent lactic acid. All were living 10 days after the last injection.

DISCUSSION

The results obtained with the B. subtilis are negative throughout. Neither the addition of lactic acid to the culture medium, nor the injection of lactic acid into mice at the time of inoculation, cause the strain of B. subtilis used by us to become virulent for mice.

The results with the B. proteus are not so clear cut. Of the six control mice injected with 1 c.c. of a 24 hour old culture, one died in 24 hours; of the six mice injected with B. proteus and lactic acid, two died; of the six mice injected with B. proteus grown on 0.08 per cent lactic acid in bouillon, one died.

The lactic acid does not seem to have increased the virulency of the B. proteus for mice. B. proteus that have been killed by heating to 58° C. for one hour had the same effect as the living strain when injected with lactic acid. The results obtained in this work do not agree with those reported by Much, but confirm the observations made by Bachmann and also those of Lange and Yoskioka.

The injection of lactic acid into a mouse inoculated with an avirulent strain of streptococcus does not increase its virulency for this animal. The results were also negative if the lactic acid is injected several days after the streptococci.

The B. proteus was the only organism used by us that was toxic for mice. The apparent increased virulency of this bacterium for mice when combined with lactic acid can be more readily explained by its impairment of the defensive mechanism of the host, than by an actual change in the biological characteristics of the microorganism causing it to become more virulent.

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CONCERNING THE INFLUENCE OF THE INTRAVENOUS ADMINISTRATION OF MERCURY BENZOATE ON THE WASSERMANN REACTION OF APPARENTLY NORMAL INDIVIDUALS*

By Albert Strickler, M.D., Philadelphia, Pa.

FOR many a generation, mercury had been the sheet-anchor in the treatment of syphilis. The discovery of chemotherapeutic methods, resulting in the introduction of arsphenamine and neoarsphenamine as specific remedies for the treatment of syphilis, have, for a time, caused the gross neglect of the mercurials. Following the subsidence of the arsphenamine wave of enthusiasm and the same appreciation of the value of organic arsenicals in the medical armamentarium, the value of the mercurials began again to be more and more appreciated.

The early use of mercury by mouth in the treatment of syphilis, a method still in vogue, to the inunction method, then, the intramuscular injection of mercury and finally the intravenous administration of this remedy, represents the various steps in the therapeutic refinements and the desire of exact medication.

The attempt on the part of some English and French syphilographers and to a minor degree, the efforts of a few American syphilographers to popularize intravenous mercurial therapy has caused us to study this problem.

In a previous communication, the author studied the influence of intravenous administration of arsphenamine and neoarsphenamine upon the Wassermann reaction of normal individuals. This study seemed to prove that these organic arsenicals were capable of influencing in a positive manner the Wassermann reaction of normal patients. The same results have also been obtained by Ravaut following similar lines of investigation.

It, therefore, occurred that it would be of value to determine whether or not mercurial preparations influenced the Wassermann reaction of apparently normal patients. With this object in view, twelve patients were selected, suffering from either one of the following skin conditions, particularly the chronic dermatoses, such as chronic eczema, lichen planus, sycosis vulgaris, etc. The treatments were given at weekly intervals and were administered intravenously.

The salt of mercury usually employed was the Benzoate and the dose varied from $\frac{2}{5}$ of a grain to $\frac{3}{5}$ of a grain. The salt of mercury was dissolved in normal salt solution. In a few instances, mercury cyanide was administered. A Wassermann reaction was performed on the serum of each patient before treatment was begun. Following the treatment, at various intervals, the complement fixing powers of their blood was tested.

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Received for publication, January 30, 1923.

A study of the following table discloses that eleven patients had a negative Wassermann reaction before mercurial treatments were instituted. One patient had a 4+ reaction at the beginning of the treatment and in spite of twenty mercurial injections, the Wassermann still remained 4+.

The number of mercurial injections varied from eight up to thirty-seven. In nine of the patients, the Wassermann reaction remained negative as a result of the intravenous injections of the mercurials. In one instance, the Wassermann reaction became ± after the twenty-second injection and then became negative when again taken after the twenty-ninth injection of the remedy.

In another instance, the Wassermann was negative before the mercurials were administered and became a 4+ after the fourth injection, remained positive 4+ after the twelfth injection and was still a 4+ after the eighteenth injection when the patient disappeared. This particular patient had an acute psoriasis of nine months' duration which involved the arms and legs.

RESUME

As a result of our study relative to the influence of the mercurials administered intravenously, upon the Wassermann reaction of apparently normal individuals, we can state:—

(1) It is our belief that the intravenous administration of the mercurials does not influence the Wassermann reaction.

(2) In our series as high as thirty-seven injections were given to a patient without producing a positive complement-fixation test.

(3) In one instance, where the Wassermann reaction was 4+ before treatment was instituted, the administration of twenty intravenous mercurial injections failed to influence the Wassermann reaction.

(4) In one instance, a patient suffering with psoriasis, developed a 4+ reaction after the fourth mercurial injection and this 4+ reaction persisted after eighteen injections of the remedy.

The effect which mercury intravenously administered seems to have upon the Wassermann reaction of apparently normal individuals, appears to differ from the effect which arsphenamine and neoarsphenamine, similarly administered, seem to exert upon the Wassermann reaction of similar individuals.

The hypothesis which we offer in explanation of our findings is as follows: It is generally admitted that the arsenicals have an affinity for the liver. This fact has been confirmed by Kolmer and Lucke in their study of the effect of the intravenous administration of the organic arsenicals, arsphenamine and neoarsphenamine upon rats and rabbits.

It is also generally believed that whatever the nature of the substance which causes a positive Wassermann may be, it is probably a substance of lipoidal nature. It would appear probable that the arsenicals in their action upon the liver cells may so alter their functions, true, probably only in a temporary fashion, as to cause either a change in the lipoids, or the lipoidal substance may flood the blood stream so that the serum, when examined at that particular time, produces a positive complement-fixation test (a positive Wassermann reaction).

On the other hand, it has been definitely established that the mercurials have a great affinity for the kidney structure. The mercurials do not profoundly influence the liver structure. As a result, the administration of the mercurials does not influence in a positive manner, the Wassermann reaction of normal individuals.

We advance this theory to explain the difference between the organic arsenicals and mercury in so far as it concerns the Wassermann reaction of apparently normal (nonsyphilitic) individuals.

CASE NO.	RESULT OF WASSERMANN BEFORE TREATMENT	TREATMENT AT WEEKLY INTERVALS	RESULT OF WASSERMANN AFTER TREATMENT
1—W. H.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 3 inj. Wassermann negative " 11 " " " " 18 " " ++ " " 29 " " "
2—J. A.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 1 inj. Wassermann negative " 4 " " " " 12 " " " " 14 " " " " 19 " " " " 21 " " " " 23 " " "
3—W. H. W.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 7 inj. Wassermann negative " 11 " " " " 19 " " " " 21 " " " " 23 " " " " 29 " " " " 33 " " " " 37 " " "
4—M. S.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 12 inj. Wassermann negative
5—P. S.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 4 inj. Wassermann negative " 8 " " " " 15 " " "
6—M. S.	+4+4+4	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 18 inj. Wassermann +4+4+4 " 19 " " +4+4+4 " 20 " " +4+4+4
7—J. E. W.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 3 inj. Wassermann negative " 8 " " "
8—P.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	" 11 " " "
9—S.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 19 inj. Wassermann negative " 23 " " " " 27 " " "
10—I. G.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 4 inj. Wassermann negative " 8 " Anticomplementary " 15 " " negative " 17 " " " " 18 " " " " 19 " " "
11—W. A.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 4 inj. Wassermann +4 " 12 " " +4+4+4 +4
12—I. L.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 22 inj. Wassermann negative

DIAGNOSTIC VALUE OF THE KAHN TEST FOR SYPHILIS*

A Preliminary Report

By Margaret S. Grant, M.D., Fort Wayne, Ind.

MUCH interest has been developed in recent years in precipitation tests for syphilis, this interest being undoubtedly due to the fact that physicians have for a long time felt the need of a laboratory test for syphilis less complicated than the Wassermann. The numerous sources of error inherent in the Wassermann test have to this day kept many physicians from depending on this test. The fact that two different laboratories are likely to give two different Wassermann results on one specimen of blood is well known. Physicians, not only in the United States, but in other countries, are therefore showing an unusual interest in simple precipitation tests with a hope that they might obtain a more correct diagnosis as well as a better check on treatment in syphilis. In Germany the Meinicke and Sachs-Georgi reactions are being discussed to a considerable degree at the present time. In England the Dreyer and Ward Sigma reaction is receiving some attention. In this country, however, the Kahn reaction is finding particular favor.

I shall not go into detail regarding the Meinicke, Sachs-Georgi and Dreyer and Ward reactions, except to say that the Meinicke and Sachs-Georgi reactions have not been accepted with favor in this country or in England. One of the main reasons for this possibly lies in the long incubation period of forty-eight hours which is required to bring out the precipitates in these reactions. This incubation frequently causes bacterial contamination, making the final reading of results quite difficult.

Several workers have reported on the Sachs-Georgi reaction in this country. Kilduffe,[1] who has made a special study of the diagnostic value of this test, sums up with the following significant statements:

1. The reaction is neither as delicate nor as trustworthy as the Wassermann test.

2. A diagnosis of syphilis or conclusions as to the results of treatment, cannot be based upon the results of a Sachs-Georgi reaction with safety, and the reaction does not seem suitable for general use for this purpose.

Craig and Williams[2] also find this test undependable for the serum diagnosis of syphilis.

Regarding the Dreyer and Ward reaction,[3] it is generally believed to be no less complicated than the Wassermann test itself. The antigen has to be prepared with unusual care, and from nine to twelve tubes must be set up for each test.

*From the Duemling Clinic.
Received for publication, Dec. 6, 1922.

Turning to the precipitation test as proposed by Kahn[4] we find an altogether different condition. The test is far less complicated than the other precipitation tests. In the case of the four-plus and three-plus reactions, furthermore, the precipitates are so marked that they can be seen without the slightest difficulty. This in contradistinction to the other precipitation tests which require an agglutinoscope to read the results. The two-plus and one-plus reactions in the Kahn test can also be seen with the naked eye after some little experience. A particularly valuable feature of this test is the so-called spontaneous reaction. After mixing the proper amounts of serum and antigen, one will frequently observe in the case of strongly positive serums a definite precipitate within a few minutes. This should be of help in special cases of blood transfusion when it is desired to eliminate active syphilis in the donor. It should also prove to be of use in emergency surgical operations when it may be necessary to know the existence of syphilis. About 90 per cent of the stronger reactions show marked precipitation in the Kahn test after three to four hours incubation. The test as a whole, however, is incubated overnight in order to bring out the weaker reactions.

A brief summary of the main points of the Kahn reaction is given by Keim and Wile:[5]

1. The patient's serum is employed in undiluted form, it having been shown by Kahn that diluting the serum with salt solution delays the rapidity of the reaction.

2. The antigen is prepared so as to contain a high content of antigenic material. This is obtained by using an alcoholic extract of dried heart muscle, which has been previously extracted with ether, thus removing fat and other nonspecific substances.

3. The antigen is diluted for the test with approximately the smallest amount of physiologic sodium chloride solution which will hold it in solution, rendering it thereby susceptible to precipitation when mixed with positive serum.

4. The antigen and serum are employed in that relationship which will, in the author's opinion, result in maximum precipitation.

We became particularly interested in the Kahn test after reading Keim and Wile's unusually favorable report on this test. These workers made an extensive study of the test at the Dermatological Clinic of the University of Michigan Hospital at Ann Arbor. Altogether they studied about three hundred fifty cases of which one hundred ninety-three were patients in various stages of syphilis. In their work each Kahn test was checked with two Wassermann tests in two different laboratories. In their cases of primary syphilis all the serums which were positive with the Wassermann reaction were positive also with the Kahn; many weak Wassermann positives proved strong positives with the Kahn; and one with a negative Wassermann gave a positive Kahn. In the cases of secondary syphilis there was the same sensitiveness noted in the Kahn reaction as compared with the Wassermann test. Their study of the cases of tertiary syphilis led them to conclude that the Kahn reaction is less sensitive in those cases than the Wassermann with prolonged

ice-box fixation, but more sensitive than the Wassermann with short ice-box fixation. In cerebrospinal syphilis the results are practically the same with both tests. In congenital syphilis the Kahn reaction gives rather more positives than even the Wassermann with long ice-box fixation, and in latent syphilis the sensitiveness is decidedly in favor of the Kahn reaction. In their series of one hundred fifty-seven nonsyphilitic cases, which consisted of various forms of dermatosis, all were negative to the Wassermann test, one hundred fifty-four were negative to the precipitation test, one was a case of acne giving a two-plus Kahn reaction, and one case of smallpox and one of diabetes, both giving a one-plus.

EXPERIMENTAL PROCEDURE

In this laboratory we have been running the Kahn precipitation test in conjunction with the Wassermann test on all serums and spinal fluids sent us, and now have one hundred seventy-five cases on which we can report findings.

In making our Wassermann test we use the human cell system, and two antigens, a plain alcoholic extract and Noguchi antigen. The latter, in our experience, is as sensitive as a cholesterinized antigen without the tendency to give false positives. Water-bath incubation is used.

In performing the Kahn test the author's[4] routine was rigidly adhered to. The serums were inactivated for thirty minutes at 56° C. Two cholesterinized antigens were used. These were diluted by adding three volumes of normal salt solution to one of antigen. To 0.3 c.c. quantities of each serum was added 0.05 c.c. of antigen; the tubes shaken for three minutes, and examined for spontaneous precipitation which might then be observed in all strongly positive serums. The tubes were then placed in the incubator for from twelve to eighteen hours, and the final readings made according to the following scheme, as designated by Kahn: A precipitate consisting of one or several large clumps, four-plus; a large flocculent precipitate, three-plus; moderate sized flocculi or granules, two-plus; small sized flocculi or granules, one-plus; fine flocculi or granules, plus-minus; no precipitate, negative.

TABLE I

	WASSERMANN	KAHN REACTION SPONTANEOUS	KAHN REACTION FINAL
153 serums	-	No	-
10 "	++++	Yes	++++
1 serum	+++ to ++++	Yes	++++
1 "	+++	Yes	+++

An important point in the preparation of the serums is that they shall be absolutely clear, otherwise the reading of the weaker reactions is not easy. Slightly opalescent serums present no particular difficulty. Anticomplementary serums, if clear, are entirely suitable for the Kahn test, since this reaction is positive or negative according to whether or not the specific reagin is present. This is an obvious advantage over the Wassermann reaction, as anticomplementary serums are not infrequently encountered in which a reading cannot be accurately made.

The accompanying table shows that in one hundred sixty-five of our cases the Kahn test checked exactly with the Wassermann.

As shown in Table II, in five cases the Kahn reaction proved more sensitive than the Wassermann. One, a treated case with three negative Wassermanns in the last two years, gave a positive Kahn at the time of the last negative Wassermann. Another case diagnosed by the physician as aortic aneurism with psychosis showed a negative Wassermann but a two-plus Kahn. In a case of carcinoma of the rectum we obtained a negative Wassermann and a two-plus Kahn. (This serum was also sent to Doctor Kahn for examination, who reported a doubtful Wassermann and a two-plus Kahn.) In an undiagnosed case the routine Wassermann was negative, Kahn two-plus. (Doctor Kahn's findings in this case also were the same as ours.) A treated syphilitic from the Feeble-Minded School gave a negative Wassermann and a two-plus Kahn.

TABLE II

	WASSERMANN	KAHN REACTION	
		SPONTANEOUS	FINAL
1. Treated case; three negative Wassermanns in past two years	–	Yes	+++
2. Diagnosed as aortic aneurism with a psychosis	–	No	++
3. Carcinoma of rectum, syphilis questioned	±	No	++
4. Undiagnosed case	–	No	++
5. Treated case from School for Feeble-Minded	–	No	++

The five spinal fluids of Table III which were submitted to this test gave rather unsatisfactory results. One, a case of epilepsy, was negative with both the Wassermann and the Kahn, as also an undiagnosed case, and a case of spastic paraplegia. A case of tabes dorsalis with a negative Wassermann, and one of general paresis, both showed merely a milkiness of the spinal fluid-antigen mixture.

TABLE III

RESULTS WITH SPINAL FLUIDS	WASSERMANN	KAHN REACTION	
		SPONTANEOUS	FINAL
1. Case of epilepsy	–	No	–
2. Undiagnosed case	–	No	–
3. Tabes dorsalis	–	Milky	Milky
4. Spastic paraplegia	–	No	–
5. General paresis	++++	Milky	Milky

It must be remembered in this connection that according to Kahn, the application of this test to spinal fluids is still in the experimental stage.

No cases of primary syphilis are included in our series, but all the syphilitics were in the later stages of the disease. The cases on which the tests were made simply as a matter of routine and found to be negative to both the Wassermann and the Kahn, include the following diseases: furunculosis, arthritis, asthma, spastic paraplegia, secondary anemia, spondylitis, neurosis, myocarditis, cholecystitis, hyperthyroidism, epilepsy, eczema, neu-

ritis, hypertension, brain tumor, sciatica, varicose ulcer, urticaria, and glandular tuberculosis.

CONCLUSIONS

1. The Kahn reaction is a simple and practical test the technic of which is easily mastered, the reading of the results requiring comparatively little experience, except in distinguishing between the weaker reactions.

2. In the application of the Kahn reaction there is less chance of error in technic than in the Wassermann since the only elements entering into its performance are antigen and patient's serum.

3. As at present developed the test does not seem of particular value in the case of spinal fluids.

4. Our results indicate that the Kahn precipitation test is a most valuable check on the Wassermann test and should be done on all serums submitted for the Wassermann.

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LABORATORY METHODS

SOME OBSERVATIONS ON THE LARGE MONONUCLEAR INDEX IN CHRONIC APPENDICITIS*

By L. O. Dutton, M.S., Memphis, Tenn.

IN the routine blood counts on suspected cases of appendiceal infections both chronic and acute, it was noticed that the large mononuclear index in the differential count was rather irregular, often showing a definite increase. As a mononuclear increase is expected only in such conditions as malaria, typhoid, etc., the following studies were undertaken to establish the frequency of such occurrences in appendiceal involvements which might prove to be a valuable diagnostic aid.

While this study was in progress my attention was called to Friedman's[1] article detailing the same kind of study, in which he established the fact that in 87 per cent of chronic appendicitis cases the large mononuclear index was greater than 5 per cent, which he took to be normal. Friedman gave figures for both the large mononuclears and transitionals, but grouped them in one total for his final results.

It has been my classification for some time to regard the mononuclears and transitionals in one group. Pappenheim as quoted by Naegeli[2] points out that the change from transitional to polymorphonuclear neutrophile, in all probability, never occurs. However the neutrophiles, eosinophiles, and basophiles arise from the same embryonic cells (myeloblast) as do the mononuclears and transitionals, and it can easily be conjectured that certain stimuli would cause an increase in mononuclear formation instead of neutrophile formation.

Indeed it is just this, presumably, that occurs in malaria, and typhoid and it seems probable that the same result occurs in certain other types of infections. In Friedman's series of cases the diagnosis of chronic appendicitis is well established in each case, and it seems certain that in this condition the large mononuclears are definitely increased. He states, however, that the increase in large mononuclears is diagnostic of this condition, of course with the proper physical findings concurring. It occurred to me, however, that the mononuclear increase might not so constantly point to lesions of the appendix, and that if such a chronic infection gives an increase of mononuclears it might be possible for the same result to occur in chronic infections of other regions. Friedman points out a number of conditions that give no increase in mononuclears, but his conclusions in this respect are based on rather meager evidence.

*From the Laboratories of the Baptist Memorial Hospital, Memphis, Tenn.
Received for publication, January 30, 1923.

To throw light on these points, and to establish the value of the differential as a diagnostic guide in chronic appendix, a study of several series of cases was made by compiling data from the records after discharge. Obviously none of the work was done under experimental conditions, and perhaps some of the value of these observations is lost thereby. All the blood counts however were done by myself, and all the pathologic examination of specimens was done by Dr. N. E. Leake, thus eliminating the varying personal element in those respects. In each case the diagnosis of the condition was arrived at by the surgeon without being aware that such a study was being made. In most cases the differential was made on 100 cells, in a few on two hundred. Of course I am aware of the attending percentage of inaccuracy in such counts; but, due to the low total count, it is very seldom expedient in routine work to include more than 100 cells in these counts.

The first study was of a series of 52 cases with a preoperative diagnosis of chronic appendicitis. Of these, 40 showed chronic changes, or acute exacerbations of a chronic appendix (two cases). Of these 40 cases 19 (47.5 per cent) showed a large mononuclear count greater than 5 per cent.

This variation from Friedman's figures is rather wide and it was thought, because of the fact that the preoperative diagnosis was not arrived at under experimental conditions, thus insuring exhaustive evidence, that some of the cases could possibly be excluded. Chronic changes were found in the specimen of all cases. But according to Gaylord and Aschoff[3] some of these apparently chronic changes might be physiologic. Excluding cases in which the pathology was of such a nature (distal end fibrosis) that it might be physiologic the figures rearrange themselves slightly. Six such cases are encountered in the series. None of these show increased mononuclears. This leaves us 34 cases of absolute chronic appendicitis with 19, or 55.9 per cent, showing a mononuclear increase. This still leaves a wide discrepancy as compared to Friedman's figures.

It is interesting to note that 5 of the original 52 cases revealed normal appendices on examination, and that 1 of these has an increase to 9 per cent large mononuclears. Four of the 52 were found to be acute upon pathologic examination of which 1 showed a count of 6 per cent mononuclears.

Thinking that if a mononuclear increase was to be taken as evidence of an appendiceal lesion, acute cases might show an increase in these cells. A study of cases was undertaken with this in view.

The second series was of 77 cases with a preoperative diagnosis of acute appendicitis. Of these 77 cases 67 proved, on pathologic examination, to be actually acute cases. Of these 67 cases 9, or 15 per cent have large mononuclear increase over 5 per cent. Four of these 77 cases proved to be chronic, 2 of them having mononuclear counts over 5 per cent. Three of the 77 proved to be normal, none having increased mononuclear counts.

It is readily seen from these cases that the mononuclear increase is not due to the appendix being infected, but rather, it is inferred, to the type of infection; as only 15 per cent of acute cases have a mononuclear increase. It is possible that the 15 per cent represent cases of long standing, the acute features

of which masked the chronic changes on examination. It seems likely from these findings, that the mononuclear increase was due to a mild stimulation by a chronic infection. To determine whether or not this increase was due to the seat of infection, a third series was studied.

In this series, 15 cases of chronic cholecystitis, uncomplicated, are contained. Seven, or 46.6 per cent of these show an increase in mononuclears. Only one out of five acute cases contained in the series shows a mononuclear increase. Two cases, which were complicated by chronic appendicitis, showed an increase of mononuclear cells.

These findings are approximately the same as found for chronic appendicitis and although based on a small number of cases certainly point to a source of future study. They also warn the diagnostician to not hastily accept a large mononuclear increase as a sign of chronic appendicitis only. A fourth series of cases was studied to see if more information could be gained on this phase of the subject.

This series contained 23 uncomplicated cases of chronic salpingitis. Of these 7 or 30.5 per cent, showed an increased mononuclear count. Two cases complicated with chronic appendices also have an increased mononuclear count.

Here again we find a chronic condition giving some increase in the mononuclears; and we are forced to conclude that the appendiceal lesion is not alone responsible for a mononuclear increase.

SUMMARY

A series of cases of chronic appendicitis were studied to determine the value of an increased mononuclear count as a diagnostic aid.

It was found that 47.5 per cent, or perhaps 55.9, excluding possible physiologic appendicitis, of the cases actually have an increase in large mononuclears. This is at wide variance with Friedman's findings. To establish whether or not any lesion of the appendix was capable of increasing the mononuclears, a study of acute cases was done showing that only 15 per cent showed a mononuclear increase.

To determine the possibility of a mononuclear increase being selective for a chronic infection of any site, rather than of the appendix only, two series were studied—(one of salpingitis and one of cholecystitis) revealing that 30.5 per cent and 46.6 per cent respectively of these cases showed a mononuclear increase over 5 per cent.

In these series the large lymphocytes were studied. In series 3 (cholecystitis) it was found that 10 or 58.8 per cent of the 17 chronic cases show high large lymphocyte counts, and this suggested the possibility of a significance of some kind. However none was established in the other conditions as the lymphocyte count was too variable.

CONCLUSIONS

1. In about 50 per cent of the cases of chronic appendicitis an increased mononuclear count is an aid in diagnosis.

2. An increase in mononuclears is in all probability not due to the appendix being infected but to the character of the infection (chronic).

3. In chronic salpingitis and in chronic cholecystitis a number of cases (30.5 per cent and 47.5 per cent) showed a mononuclear increase.

4. The diagnostician should not rely too heavily in doubtful cases on the findings of increased mononuclears as a sign of chronic appendicitis, but should bear in mind the possibility of at least two other conditions giving the same blood picture.

It is to be borne in mind that this study was done under routine conditions and therefore is not to be taken as an experimental study but rather a survey of routine results.

Further studies are being done, on an experimental basis, to more securely establish the value of the above observations.

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THE EMPLOYMENT OF VEGETABLE EXTRACTS IN THE WASSERMANN REACTION*

By Emil Weiss, M.D., Chicago, Ill.

THE antigens used in the Wassermann reaction have been obtained, as a rule, from animal origin. The historical review of this subject has been covered in detail by several authors. We wish only to mention those publications that have a direct bearing on our problem.

Tribondeau[1] extracted material from vegetable origin and obtained reliable antigens as compared with those from animal origin. This author used peas, beans and corn. These were ground up in a mortar, washed with absolute alcohol, acetone or ether, then centrifuged, the supernating fluid was decanted off and the sediment resuspended; this process was repeated until the supernating fluid was clear. The vegetable was then dried and extracted with alcohol or acetone and concentrated by evaporation. This author found the antigens from peas gave the most consistent results. Heinlein[2] extracted an antigen from potatoes. The peeled and grated potatoes were treated with five times their volume of alcohol. From this step on the technic was the same as usually employed in the preparation of organ extracts. Heinlein found that the potato antigen only gave positive results with some of the syphilitic sera. This author also found that the addition of cholesterine did not give a larger percentage of positives as compared with the same procedure with his control animal extracts. This potato antigen deteriorated quickly, sometimes after two weeks standing it was not usable.

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Received for publication, January 30, 1923.

The writer thought it worth while to prepare extracts from a series of vegetables and carefully test these extracts as to their usefulness as antigens for the Wassermann reaction.

The majority of serologists at the present time use several different antigens in the technic of the Wassermann reaction. It would be advantageous to be able to use, in a confirmatory way, antigens from vegetable as well as from animal origin. In addition to the vegetables being useful for preparation of the extracts, they are very easily obtained. The procedure of the extraction is very simple.

TECHNIC OF PREPARATION OF ANTIGENS

Antigens were prepared from the seeds of the following vegetables, viz: ricinus, poppy, rape, hemp, flax, squash, watermelon, sunflower, radish, mustard, beans, barley, corn, peas, lentils, and from the meat of walnut, cocoanut and olive.

All foreign substance was removed and the material was then ground up into very small particles and dried, then two volumes of alcohol or acetone added and placed in a shaking apparatus for twenty-four hours. After this, the flasks were set aside at room temperature for five or six days. If a shaking apparatus is not at one's disposal, it can be kept for ten or twelve days at room temperature, but must be shaken up several times a day. The contents of the flasks are then filtered through a double layer of hard filter paper, the clear extract was transferred to amber colored bottles and kept at room temperature. It is better to keep these antigens at room temperature than to keep them in the ice box at a lower temperature. If the extracts are cooled to a lower temperature after they are prepared, weak extracts have a tendency to become cloudy and stronger extracts show sediment. It is possible to clear up the cloudiness and redissolve the sediment by gently heating the flask. This can be eliminated if the ice box is not used to preserve the antigens.

THE EXAMINATION OF EXTRACTS

Kaup[3] gave a very accurate method for the titration of the proper amount of antigen to be used. This author determined the different zones of each antigen in the following way:

(1) The extract, amboceptor and sheep cells suspension.

(2) Extract, normal serum, amboceptor and sheep cells suspension.

(3) Extract, complement, amboceptor and sheep cells suspension.

(4) Extract, normal serum, complement, amboceptor and sheep cells.

(5) Extract, syphilitic serum, complement, amboceptor and sheep cells suspension, varying the amount of antigens from .5 c.c. to .0001 c.c.

According to Kaup's method, with a useful extract one distinguishes from the largest down to the smallest doses, the following reactions: A large amount causes a precipitation of albumin, (precipitation zone), a slightly smaller amount causes hemolysis, (hemolytic zone), and in still smaller doses shows inhibitions, (inhibitions or anticomplementary zone), a smaller amount shows

TABLE I

A.

SIMULTANEOUS PERFORMANCE OF THE WASSERMANN REACTION WITH NORMAL AND OLIVE EXTRACT.

NUMBER OF EXAMINED SERA:	NUMBER AND PERCENTAGE OF SERA WITH SAME RESULTS	NUMBER AND PERCENTAGE OF SERA WITH DIFFERENT RESULTS	ORGAN EXTR. C.I.: OLIVE EXTR. C.I.:	ORGAN EXTR. C.I.: OLIVE EXTR. I.I.:	ORGAN EXTR. I.I.: OLIVE EXTR. C.I.:	ORGAN EXTR. I.I.: OLIVE EXTR. I.I.:	ORGAN EXTR. I.I.: OLIVE EXTR. L.:	ORGAN EXTR. L.: OLIVE EXTR. I.I.:	ORGAN EXTR. L.: OLIVE EXTR. L.:
1183	1099 = 92.9%	84 = 7.1%	301	28	35	84	9	12	714

B.

SIMULTANEOUS PERFORMANCE OF THE WASSERMANN REACTION WITH NORMAL AND COCOANUT EXTRACT.

NUMBER OF EXAMINED SERA:	NUMBER AND PERCENTAGE OF SERA WITH SAME RESULTS	NUMBER AND PERCENTAGE OF SERA WITH DIFFERENT RESULTS	ORGAN EXTR. C.I.: OLIVE EXTR. C.I.:	ORGAN EXTR. C.I.: COCOA. EXTR. I.I.:	ORGAN EXTR. I.I.: COCOA. EXTR. C.I.:	ORGAN EXTR. I.I.: COCOA. EXTR. I.I.:	ORGAN EXTR. I.I.: COCOA. EXTR. L.:	ORGAN EXTR. L.: COCOA. EXTR. I.I.:	ORGAN EXTR. L.: COCOA. EXTR. L.:
1031	938 = 91%	93 = 9%	231	29	38	77	11	15	630

c.I.=complete inhibition.
i.I. =incomplete inhibition.
L. =hemolysis.

the specific reaction (specific or antigenic zone) and the smallest dose shows hemolysis, (or inefficient zone). This method, with minor modifications, was used for the examination of the vegetable extracts. The vegetable extracts, examined in this manner, gave the same reactions in the various zones as was found in different organ extracts.

In order to show the influence that the extracted substances alone have on the different zones, we evaporated carefully an alcoholic olive extract at low temperature to one-fourth of its original volume, this was then made up to volume with normal salt solution. This extract showed the same reaction in the different zones as the original extract, the only difference being that a slightly larger quantity had to be used.

1 c.c. of a 1 per cent solution of cholesterine was added to 10 c.c. of an alcoholic olive extract. This cholesterinized vegetable antigen was made stronger in the same way as an animal extract.

CONCLUSION

The vegetable extracts examined by us as to their usefulness as antigens in the Wassermann reaction, can be divided into three groups: Group 1, olive and cocoanut. The green olives are far better than the ripe ones. The extracts from these are as useful as the extracts from animal tissues. The antigenic or specific zones are clear cut and reliable. The only difference in the alcoholic and acetone extracts is that with the latter the zones are shifted a little upwards, that is, that same reaction but a little larger amount of the extract is required to parallel the alcoholic extracts. The differences in the results between the normal animal extracts and these two vegetable extracts were never greater than one always finds between two different animal extracts. The vegetable extracts never showed a negative reaction where an organ extract showed a complete inhibition, and the vegetable extracts never give strong inhibition when the organic extracts show complete hemolysis. (Table I.)

Group II. Ricinus seed, flax seed, hemp seed, radish seed, sunflower, poppy seed, rape seed, peas and mustard seed. With these extracts the antigenic or specific zone is very narrow, the difference between positive and negative sera is not great enough to be used as a reliable antigen in the Wassermann reaction.

Group III. Corn, walnut, beans, barley, watermelon seed, squash seed and lentils. The extracts of this group of vegetables are absolutely useless as antigens for the Wassermann reaction. There is no difference between positive and negative sera, the specific or antigenic zone does not exist.

SUMMARY

The alcohol or acetone extracts of olive and cocoanut are very useful as antigens for the Wassermann reaction. The difference in the results between vegetable and animal extracts is no greater than that between two different animal tissue extracts. The addition of cholesterine increases the breadth of the specific zone the same as with animal extracts. For the extraction of

vegetables, alcohol, 95 per cent or absolute and acetone, are equally useful. The durability of the vegetable extracts at room temperature is the same as that of the animal tissue extracts.

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WASSERMANN SYSTEMS*

By Dr. Albert Faller, Cincinnati, Ohio

THE technic of the original Wassermann test did not concern itself with the problem of systems; it demanded sheep's corpuscles and it was not until some time later, when various vulnerable points in the test were beginning to manifest themselves, that it occurred that one of the weak points might be in the hemolytic system; it was not long after this suspicion arose until every imaginable kind of corpuscle had its supporters as being the ideal one for complement-fixation work, and this usually without defining the particular weakness of the one or the superior points of the other.

What criteria shall be accepted as indicating the system of choice? First and foremost, I should say that system is best for which the most potent amboceptor can be prepared; secondly and of almost equal importance in my opinion, is the selection of a system for which the particular serum to be tested contains the least amount of lysin; thirdly, we must consider the availability of corpuscles; fourthly, we must consider the possibility of more or less isohemolysin and fifth and of rather minor importance is the question of clarity of contents of our tubes.

This paper is based upon the investigation of those animals which would enable us to meet the requirements of the third criterion—those bloods most available, namely, sheep, beef, human, chicken, duck, goose, guinea pig, and rabbit.

One of the earliest and most popular deviations from the original Wassermann technic was the employment of the human blood system as popularized by Noguchi; this system has among its recommendations the fact that it is sponsored by this brilliant worker; also in its favor is the fact that nothing is cheaper or more available than human blood; the use of amboceptor papers in this system is said to be an advantage. Weighed against these advantages is the fact that an antihuman amboceptor of high titer cannot be produced and the writer feels that rapidity and clearness of action in the second incuba-

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Received for publication, February 5, 1923.

tion permits of the exercise of a great deal of judgment in interpreting readings. Wm. C. Williams[1] has shown the dangers of error when the human system is used, resulting from the presence of antihuman hemolysin in guinea pig serum, specific for corpuscles of groups one and two. The same author maintains that iso-hemolysins for human cells exist in human serum, necessitating the typing of corpuscles used in the test. Such sources of error would tend toward false negatives; of even graver importance is the fact that, using the human system, a considerable number of false positives are encountered. Some years ago, before I had investigated the adaptability of the various systems, while the slaughter houses were idle as the result of the prevalence of foot and mouth disease, I was glad to avail myself of the antihuman system in spite of its faults. Today idle stock yards would cause me but little concern.

Of the different fowls used in complement-fixation work, my experience is limited to the use of the blood of the chicken, duck, goose, and guinea fowl; these all are fairly adaptable under the requirements relative to the potency of artificial amboceptor, but the great majority of human sera are rich in natural amboceptor hemolytic for these corpuscles and in this respect they offer no advantage over the sheep system; also militating against their use is the fact that it is very difficult to wash the corpuscles of fowls to the point where they give a perfectly clear picture in negative tubes; there seems to be something in nucleated corpuscles which gives more or less of a murky fluid after hemolysis of corpuscles which is sometimes very annoying in reading end results. In their favor, of course, is their usual availability. It is very comforting to know that systems of such utility, possessing no greater disadvantages of a serious nature, are always at hand if an emergency should arise.

Grouping the sheep, ox, hog, and guinea pig, they all meet requirements in about equal measure, producing amboceptor in the rabbit of fairly high titer; against them in almost equal measure, I have found that human serum contains great amounts of natural amboceptor, being greatest in antiguinea pig and lowest in antiox, but the difference is so small as to be of no practical consequence; however, individual sera vary considerably against individual corpuscles; one serum may be rich in antisheep amboceptor and poor in antiox or antiguinea pig, and in doubtful cases we select our system accordingly; our greatest reliance is placed in sheep, ox, and guinea pig, the latter, because, like the poor, it is always with us; we have one amboceptor specific for three systems; we are very partial to the rabbit also, but for obvious reasons, our one amboceptor has not been made to include this animal.

While this paper has to do only with systems, the proper pursuit of the subject must of necessity lead into the by-path of some discussion of methods of complement fixation. That a biological test be of most practical utility, it is necessary that it be as free from error as possible, but since all such tests are more or less subject to error, it becomes essential that all such sources be known; that provision may be made for their recognition and interpretation and when such errors are inherent and cannot be remedied, it becomes necessary to adopt a substitute test or a modification which is free from such error.

The causes of Wassermann error inherent in the test itself are 1, presence

of but few antibodies, this resulting from (a) heating of serum; (b) effect of treatment; (c) early stage or so-called latency of the disease, 2, presence of natural antisheep amboceptor in quantities sufficient to destroy the proper ratio of Wassermann factors. Noguchi[2] has shown that during the first five minutes of inactivation about 49 per cent of luetic antibodies are destroyed; during the next five minutes 20 per cent disappear and at the end of thirty minutes inactivation but 25 per cent remain. Thus the necessity for conserving antibodies, especially in intensively treated and in early and latent cases, becomes apparent; and as these form a great part of the cases coming to us for diagnosis, it is equally apparent that a large number of these cases do not readily lend themselves to the unmodified Wassermann technic, limiting itself as it does, to the antisheep system. The second great source of error is the presence of antisheep amboceptor in quantity sufficient to destroy the proper ratio of Wassermann factors. Excess of amboceptor is disconcerting only when antibodies are few. It is my experience that a serum containing enough natural antisheep amboceptor and luetic antibodies to cause a four-plus reaction, shows no hemolysis when considerable amboceptor is added, but frequently does show effect in less strongly positive cases; it is the loss of balance between amboceptor, in excess and antibodies deficient in amount, that causes negative reactions where positive ones should obtain; the smaller the amount of antibodies, the more probable that excess of amboceptor will destroy a delicate balance. Where antibodies exist in great numbers a comparatively rough approximation of reagent quantities may be tolerated, but in the presence of small amounts a most delicate manipulation is imperative. Most human blood contains enough amboceptor, either antisheep, ox, hog, rabbit, guinea pig, chicken, duck, goose or guinea fowl, one, more or all of these, to cause hemolysis in very considerable amount. I have frequently seen sera capable of causing hemolysis in proportion of .1 c.c. serum to 1.5 c.c. of corpuscles in 5 per cent suspension. The complement of human serum, while usually not in great amount, is nevertheless quite constantly present and increases the effect of the natural amboceptor. The Wassermann technic, while demanding careful titration of amboceptor and complement added to the test, takes no cognizance of the amount of these factors naturally present in the serum; it does not seem reasonable that a serum containing a great amount of amboceptor, should have added to it the same amount of this ingredient demanded by a serum containing little or none of this body. It is obvious that each serum to be examined should be titrated as carefully as any other ingredient of the test; each serum should be individualized; it should be carefully titrated for its specific amboceptor and complement content; the presence of anticomplementary substances should be sought, and a comparative estimation of antibodies should be made.

Of all complement-fixation tests, the writer considers the Hecht-Gradwohl test the most widely applicable and useful; this method is useful not only as a complement-fixation test, but because of the amount of knowledge it yields concerning the peculiarities of each serum; it indicates antibodies; it shows the presence of anticomplementary substances and the kind of amboceptor, and complement present; armed with this knowledge, the serologist is in position

to properly interpret the significance of the various tests, and to select the one best suited to the serum to be tested.

The Hecht-Gradwohl test makes use of the natural antisheep or other amboceptor and natural complement and is performed with unheated serum; it is therefore designed to overcome the great sources of error encountered in the original Wassermann test.

The technic as worked out by R. B. H. Gradwohl of St. Louis is as follows: 14 tubes are placed in a rack; into each tube is placed .1 c.c. of the patient's serum; then into the first ten tubes is placed descending amounts of normal salt solution; in the first tube .9 c.c. descending to .1 c.c.; then in these ten tubes are placed ascending amounts of sheep's corpuscles, beginning with .1 c.c., and ending with 1 c.c.; this gives the first ten tubes equal volume. These ten tubes are for the purpose of obtaining the hemolytic index—the hemolyzing power of the serum. In the next three tubes are placed graded amounts of acetone insoluble antigen, .2 c.c.—.15 c.c. and .1 c.c. this diluted to one-half the Wassermann strength. The last tube contains only serum and is the control tube. These last four are then brought to equal volume with salt solution; the rack is then placed in the water-bath at 37° for one-half hour, and frequently shaken. That tube which shows complete hemolysis of the greatest amount of sheep's corpuscles is then noted and gives the hemolytic index. Sheep's corpuscles are then added to the last four tubes according to this index; if this is from 1 to 4, one-tenth c.c. of corpuscles is added; if from 5 to 7, fifteen one hundredths and if from 8 to 10, two-tenths c.c. The rack is then placed in the water-bath and results read as in the Wassermann test, when the control tube shows complete hemolysis.

It will be noted that the above technic calls for the antisheep system; antisheep amboceptor is rather frequently absent from human serum; for such contingency we keep in the ice box corpuscles of ox, rabbit, guinea pig and the several fowls and it is the work of but a few minutes to find which system lends itself to the balking serum. I remember no case where at least one system could not be applied except in infants and in those rare instances where failure of hemolysis is due to the absence of natural complement; in such cases this is supplied and the test proceeds. In our routine work we run all sera, using the Hecht-Gradwohl and Wassermann tests, using sheep system of corpuscles. If we find no antisheep amboceptor present, or if in only small amount, we know that is the ideal system for the Wassermann test as one great source of error does not exist; we then substitute that system for the Hecht-Gradwohl test for which the greatest amount of amboceptor is present and proceed as with the Gradwohl technic. We rarely need resort to fowl blood.

If the Hecht-Gradwohl test is positive and the Wassermann negative (the reverse can never be true) we consult the hemolytic index; if this is high we repeat the Wassermann, using a system for which the serum shows a low amboceptor content, employing both heated and unheated serum.

These two tests, using only the antisheep system, disagree in about 10 per cent of all cases and in most cases, at some stage of treatment; by manipulating

the systems as just described, they are made to practically approximate in results, except in treated cases.

CONCLUSIONS

1. For complement-fixation work there is no one universally adaptable system. The amboceptor content of the serum to be examined determines the system most suited.

2. That system for which the serum to be tested contains the least amount of natural amboceptor is best suited to the Wassermann test.

3. Conversely, that system for which the greatest amount of amboceptor exists in the serum to be tested is best suited to the Hecht-Gradwohl test.

4. Most human sera contain a great variety of amboceptors in varying amounts.

5. Most sera contain a considerable amount of some one amboceptor, and are poor in a certain one, indicating the system best suited to it for Wassermann and Hecht-Gradwohl tests.

6. One specimen of rabbit serum may yield amboceptors specific for several systems.

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The Journal of Laboratory and Clinical Medicine

VOL. VIII ST. LOUIS, MO., APRIL, 1923 NO. 7

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Chicago

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EDITORIALS

Uremia

THE daily clinical use of the term uremia has produced an easy familiarity and we are inclined to believe that in using it, we refer to a process well understood by all. On the contrary, the farther the study of uremia progresses, the more convinced we must become that we actually know amazingly little of the pathological condition. When the term was first adopted, the impression was, as the name implies, that the symptom complex arises from non-excretion of the normal urinary substances, and the retention particularly of urea in the blood. Hewlett fed himself urea, producing an unusually high urea concentration in the blood, without at the same time establishing any of the clinical symptoms of uremia. This, and the work of many others, established the error of the earlier conceptions. Today we still visualize the disease as secondary to and the result of severe kidney disease.

The most recent interpretation is that uremia is not due to primary renal damage, but is a general metabolic disturbance with resulting damage to many organs and tissues, among which there happens to be the kidney. In acute nephritis with edema, resulting from infection or other cause, we have usually understood a primary renal pathology. But here a change has oc-

curred which affects most of the cells of the body, presumably accompanied by alterations of the surface tension of fluids and of the permeability of cells. The renal epithelium is affected, but to no greater degree than the other tissues. As Marriott points out, these patients are not edematous because the kidneys fail to excrete water, but because the tissues hold the water and deliver little to the kidneys for excretion. This form of so-called nephritis follows damage by cellular poisons, which are often of the nature of bacterial toxins. With the removal of such poisons recovery is often complete, and the residual damage is no greater in the kidneys than in other body tissues. The frequency of acute nephritis following accessory sinus infection with complete recovery after removal of the original source of poisoning is but an example.

Interstitial nephritis is a true inflammatory disease of the kidney with resulting fibrous changes, but other forms, such as the acute nephritis just mentioned, are often systemic diseases, in which the part played by the kidney is incidental to the wider picture. According to this conception, uremia does not come from retention of poisonous or non-poisonous excretory products, but from general tissue damage by a specific metabolic poison. This poison presumably affects the kidneys at the same time that it is causing damage elsewhere.

Riesman remarks, "Of the important condition we call uremia so little is actually known that it constitutes an opprobrium of clinical medicine. Like the cause of cancer it is an unsolved riddle. We can add almost nothing today to the definition formulated many years ago by Bouchard; uremia is caused by a complex poisoning in which share, in unequal proportions, all the poisons introduced into the system or manufactured by it." Hamman defines uremia as the term used to designate a group of intoxication symptoms associated with renal insufficiency. This is as concise and as complete a definition as can be advanced in the present state of our knowledge.

It is true that uremia is the end result of nephritis but not all cases of nephritis end in uremia. Nephritis, as we understand the term, includes quite a variety of clinical entities and even in individual members of this general group, death may occur from a variety of causes.

Riesman points out that although the great multiplicity of symptoms may be thought to argue against a unitary cause for uremia, there are good reasons for the belief in a single primary poison. Whatever the nature of this poison or uremotoxin, whether it be a toxalbumin or a nonprotein poison, whether it be a crystallizable substance such as suggested a few years ago by Foster, we do know that it has a chemical affinity for many tissues and organs. This tropism is easiest recognized in its action on the liver, the central nervous system, the cardiovascular system including particularly the capillaries, the neuromotor mechanism, the adrenals and the kidneys themselves. Whatever the nature of the poison, it interferes with the normal functioning of these various structures. The multiplicity of lesions and the varying susceptibility of individuals explain in part the multiplicity of symptoms recognized in cases of true uremia.

Riesman classifies these symptoms under several headings, as follows: nervous manifestations, including aphasia, monoplegia, hemiplegia, headache, disturbances of vision and of hearing, neuralgia, convulsions and coma; mental or psychologic manifestations; visceral manifestations, almost countless in variety, but usually associated with cardiovascular changes, often with respiratory symptoms such as paroxysmal dyspnea and acute pulmonary edema, or gastrointestinal symptoms as vomiting, hiccough, and diarrhea; or urinary symptoms, observed in the clinical laboratory; dermal manifestations, such as pruritus, "dead fingers," pasty or so-called "cold buckwheat cake" appearance to the skin; and, finally, mixed manifestations, which may appear in almost countless variety. He speaks particularly of terminal pericarditis and of fever associated with uremia.

He describes the motor disturbances, particularly monoplegia, hemiplegia, and asphasia, as usually transitory and functional in character, although indistinguishable while present, from those produced by such organic changes as thrombosis or hemorrhage. Such cases at autopsy, showed no lesion, other than advanced kidney disease, which could have been held responsible for the paralysis. We have no clear knowledge as to the manner in which these transitory functional brain disturbances are brought about. It is possible that the poison acts directly on the brain cells, or it may act primarily on the blood vessels, causing spasm and cerebral ischemia.

Hamman takes exception to the inclusiveness of Riesman's enumeration of uremic symptoms. He defines the classical symptoms as lethargy, gradually progressing to coma, increased reflexes and muscular twitchings, stertorous breathing and often hyperpnea, anorexia and vomiting, a urinous odor to the breath, headache, increase in blood pressure and a predisposition to certain infections, chiefly stomatitis, enteritis and pericarditis. Convulsions, transient cerebral symptoms, psychic disturbances, paroxysmal dyspnea, pulmonary edema, Cheyne Stokes breathing and such other symptoms are often associated with uremia, but they occur still more frequently in the absence of renal insufficiency and are not necessarily due to uremic intoxication. This latter group, associated particularly with vascular changes, Hamman classifies as pseudoremic symptoms. They occur chiefly in association with hypertension and are often preceded by increases in blood pressure, the so-called blood pressure crises.

W. Langdon Brown classifies uremic symptoms as (1) cerebral, (2) respiratory, (3) gastrointestinal, (4) cutaneous, (5) muscular. He attempts to simplify this grouping. Thus the gastrointestinal symptoms, such as nausea, vomiting and diarrhea, although possibly sometimes central in origin, may result from attempts to find alternative channels of excretion. Eight grams of nitrogen may be excreted daily through the bowels. Canti, in a severe case of uremia, found the urea concentration in the blood to be 0.3 per cent, while in the vomitus it was 0.6 per cent. The skin manifestations may result from a similar process and myoclonus may be due to local intoxication as well as to a central disturbance. Thus Brown attempts to restrict the uremic syndrome to cerebral and respiratory manifestations.

He differentiates between uremia from chronic partial impairment of excretion, and urinemia, following complete suppression as from bichlorid of mercury or phenol poisoning. Urinemia is manifested by bodily weakness and languor, with few other symptoms. The symptoms of uremia do not occur in these cases, according to Brown, because fatal termination is so rapid that not sufficient uremic poison accumulates in the system. He suggests that incomplete excretion may lead to altered metabolism with the formation of abnormal toxic products, and defines uremia as a disease of metabolism, resulting from damaged kidneys. The cause of diabetic coma is not the retained or increased sugar of the blood, nor is the uremotoxin a retained urinary element. In both diseases the terminal symptoms are due to profound metabolic disturbances with the formation of a new poison. Golla suggests that in uremia the altered metabolism leads to the formation of trimethylamine, which he found increased tenfold in the blood in uremia, and that the hypothetical poison is closely related to trimethylamine. This view is not universally accepted.

Brown differentiates three forms of the acute nephritides. The first or toxemic kidney is distinguishable from a true nephritis by the fact that the urine rarely contains polymorphonuclear leucocytes, that the ratio between albumin and globulin is two to one as contrasted to six to one in true nephritis, that the output of diastase in the urine is low in nephritis but high in toxemic kidney, and that the blood urea is raised in the latter but not in the former. The blood sugar tends to show similar variations. In the toxemic kidney we have to deal not so much with a retention of excretory products as with an increased permeability of the kidney to anything which the blood delivers to it.

In the second form, or true acute nephritis, leucocytes are usually present in the urine and other differences are as stated above. The disease is often associated with infection elsewhere. Renal permeability tests show retention of chlorides and of urea and a diminished diastase output. Rarely did uremic manifestations occur in this type of nephritis.

The third form of acute nephritis or focal embolic nephritis is due not merely to the local action of a generalized toxin but to the actual deposition of infective microorganisms in the kidney. Here again, uremic symptoms rarely occur.

Among the classes of chronic nephritis, Brown calls attention particularly to what he terms "leaky kidney," a kidney which has suffered from an earlier but nonprogressive lesion. It allows proteins to escape into the urine but does not retain those substances which should be excreted and there are rarely associated cardiovascular changes. The ratio of albumin to globulin is more apt to be that found in toxemic kidney (2–1) than that of true nephritis (6–1). Renal permeability tests and the diastase output are normal. Uremic symptoms do not occur in leaky kidney, and the disease is compatible with fairly normal health despite the large amount of protein in the urine.

The point which Brown emphasizes in this discussion is that it is not the material which escapes but that which is not excreted which leads to uremia.

In an individual bordering on uremia or one in which the uremic poison is at work, sudden fluid depletion may so increase the concentration of the poison as to precipitate uremia. Thus in one case a diarrhea rapidly terminated in uremia. Water intake insufficient to maintain an adequate blood volume, when the kidney is incapable of excreting a concentrated urine may also predispose to uremia.

Brown states elsewhere that the symptoms of uremia may occur under conditions in which the kidney is not retaining toxins, such as in the toxemic kidney. This is somewhat at variance to his preceding statement. He suggests that it may be that the liver, through its inability to properly destroy the uremic poison, plays a greater part even than the kidney, in the production of uremia.

With regard to the suggestion that the drowsiness of uremia may be attributed to the acidosis that follows failure to excrete acid sodium phosphate, analogous to the acidosis of diabetes, criticism is made that even in diabetes it is not the acidosis *per se* that causes coma. The symptoms in the latter disease have been shown by Hurtley and Trevan to be due to the enolic group CO-COH, present in diacetic acid and they have shown that neutral sodium diacetate will produce the symptoms of diabetic coma equally well.

Geoffrey Evans' conception of nephritis may be summarized as follows: The kidney may be affected by pathogenic agents of varying kind and intensity, all of which, however, are capable of inducing active inflammatory reaction in the tissues they attack, whether epithelial or vascular. When acute, the epithelial reaction dominates the picture; when chronic, both epithelial and vascular changes co-exist; in the very chronic, only the vessels suffer, the pathogenic agent being too feeble to evoke reaction in the epithelial tissues, or these tissues being too feeble to react, they simply atrophy or degenerate. From this point of view neither vascular nor renal disease is to be regarded as the cause of the other, but both are due to the action of the same toxic agent.

From this partial review of work in this country and abroad, we observe that the conception of nephritis and uremia is changing from that of a local anatomical and functional disease, to that of a general metabolic disturbance in which the renal and urinary findings are secondary to a more profound intoxication. There appears still to be considerable uncertainty in the minds of those interested in the subject, as to whether the metabolic change producing uremia is incited by a primary kidney disease, or whether the renal pathology is entirely secondary to an antecedent generalized poisoning.

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—*W. T. V.*

Proposed Changes in the Medical Curriculum

AT THE recent annual conferences of the American Medical College Association and the Council on Education and Hospitals of the American Medical Association certain proposed changes in the medical curriculum were discussed. No definite action, so far as I can ascertain, was taken. However, numerous advocates of the proposed changes were in evidence. The first proposition is founded upon the belief that medical students, especially during the first two years of the course, are worked too hard. From many years' experience as a teacher in a medical school I am inclined to the belief that there is truth in this proposition. The question is whether it would be beneficial to medical students to lessen their labors during these years. Suppose that the number of required working hours is reduced, to what extent would the students be benefited by such reduction? Students now entering our best medical schools may be divided into two classes. First, those who are unable, for various reasons, to comply with the strenuous demands made upon them. My experience is that this class makes up from thirty to forty per cent of those matriculating. It is highly advantageous to the good students that these "lame ducks" should be thrown out early in the course. Would this happen if the required hours were greatly reduced? I believe that first-year medical students should be submitted to a most strenuous test and that all halt, lame and generally incompetent should be discarded, preferably by the end of the first semester, certainly by the end of the first year. The second class of our present-day medical students consists largely of those who want to work all the time. It is difficult to drive them out of the laboratory. They desire to work more hours every day than the rules permit. They are excluded with difficulty on Saturday afternoon and some of them find excuses for admission on Sunday. The most serious objection that I see to reducing the required hours in the first year, or the first two years, lies in the fact that the unfit will be continued in subsequent years and many of them will ultimately be allowed to graduate.

Another proposition put forward at the meetings referred to, advises that clinical teaching be advanced to the second and even to the first year. The advocates of this proposition hold that a deeper practical interest in medicine on the part of the student will be secured in this way. At least one proponent of this plan speaks of it as an innovation—as something new in the manner of teaching medicine. This certainly is not true. Recent explorations establish the fact that Esculapius was not a wholly mythical personage, that he lived about thirteen centuries before Christ, that he and his followers established hospitals such as that unearthed on the Island of Cos, and that these were used as medical schools. In the temples of Esculapius young men prepared themselves for the medical profession by studying under teachers the symptoms and treatment of disease in the hospital patients. The clinical teaching of medicine is as old as the profession itself. In the temple of Esculapius at Cos the young man seeking admission to the medical profession had no instruction in chemistry, biology or physics, except that he did get some

information about mineral and vegetable drugs. Under this system, medicine was a trade, not a profession. Students were apprentices, and this system of teaching medicine continued until the latter part of the nineteenth century. During all this time clinical medicine predominated in medical schools. There was no requirement of courses of science set for the matriculating medical student. In the late seventies I spent two years in a medical school. I attended clinics from the first day of entering the school. In the school which I attended there was at that time unusual attention given to chemistry, physics, and biology; indeed, this special school was held up by its admirers as particularly efficient in the scientific branches, and by the mass of medical teachers in this country it was condemned on account of the paucity of its clinical material. The majority of the students entering this school at that time had no knowledge of the fundamental facts of chemistry, physics, and biology. Compared with other schools of that time its students had unusual laboratory opportunities. Still, clinical medicine dominated the curriculum and the first-year student spent a large part of his time in having patients presented to him and their symptoms and treatment discussed before him. Certainly the proposition to advance clinical teaching to the freshman year cannot be considered an innovation. Clinical teaching without previous scientific knowledge is as old as the profession of medicine; indeed, as I have already stated, there was no profession, there was a trade. The medical student acquired what skill he could by observation on patients. Some of these students developed into skilled practitioners, just as some artisans become highly skilled workers in wood and in metal. In the first week of my course in a medical school an able clinician presented a patient, with the following statement: "Gentlemen, this is a case of ascites. Ascites is an accumulation of fluid in the abdominal cavity and may be due to disease of the heart, liver or kidneys." Looking over the shoulder of the student who sat in front and below me I read his notes, which ran as follows: "Ascites is an accumulation of hearts, livers and kidneys in the abdominal cavity."

At the recent conference of the Council on Medical Education an advocate of the plan of advancing clinical teaching to the first year said that it would be greatly to the interest of the student to bring before the class cases of tuberculosis, kidney disease, and diabetes,—that such a presentation would give to the freshman student a deeper, more direct, and more intense interest in medicine than he now acquires in the laboratories of bacteriology, physiology, anatomy, histology, pharmacology, etc. Imagine the great and clear insight into medicine that would be acquired by presenting to the freshman medical student a case of diabetes. The clinician would say, this is a case of diabetes. Diabetes is usually due to some derangement of the pancreas. The special part of the pancreas affected in the development of diabetes is to be found in the islands of Langerhans, etc. The student who does not know whether the pancreas is behind the ears or in the ankles, who does not know whether it is composed of bone or muscle or mixed, who has no conception of the islands of Langerhans, would be greatly benefited, would he not, by this talk. In my opinion, this proposition to advance clinical teaching to the

first year is absurd in the highest degree unless certain other changes are made. It is possible to demand knowledge of the fundamental medical sciences for admission to the medical schools. This is possible, but we have heard no proposition to do this. Besides, it is not likely that this will be done. It is, of course, possible to advance the requirements for admission to medical schools to three or even four years in college. This is a proposition which has two sides to it and which may be worthy of discussion.

According to my understanding, modern medicine consists in the application of certain discoveries in physics, chemistry, and biology to the prevention or treatment of disease. Modern clinical medicine cannot be taught to those who are ignorant of these fundamental sciences. An hour or two a week of clinics given to first-year medical students would doubtlessly greatly interest them. I have my serious doubts as to the benefit that either student or patient would receive from these clinics. Admitting that medical students of the first and second years are overworked in the laboratories, need some change and recreation, and would enjoy a break in the day's work, I suggest that an hour a day be devoted to vaudeville. No doubt the students would be pleased, patients would not be harassed, the time of the clinician would not be lost, and the student would return to his laboratory work refreshed. Besides, this would not involve the return to the old medical curriculum hoary with three thousand years of age. The assumption on the part of those seeking to establish clinical teaching in the first year of medicine seems to be that the present medical curriculum, which has been tried for not more than thirty years, has proved a failure. In my opinion, the scientific teaching of medicine in which the student first becomes acquainted with the facts of physics, chemistry, and biology has advanced the profession more than did the three thousand years during which the whole of the medical curriculum consisted of clinical teaching. While we still have many incompetent physicians, I believe there was never a time in the history of the world when our profession could number in its membership such a large proportion of competent and efficient practitioners. However, I should not take this matter seriously because I do not apprehend that the so-called innovation in the medical curriculum, provided it is adopted, will accomplish anything more than to waste one or two hours of the time per week of the first and second-year medical student.

—*V. C. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

*Coleman's Materia Medica for Dentists**

THIS is a small book and therefore cannot be comprehensive. As a matter of fact, it is really a compend, tabulating the characteristics and usages of each drug. Considered as such it is very good, but it would seem that even for the use of dental students the action of certain drugs at least, should be discussed in much greater detail.

At the end there are chapters on vaccine therapy, organo-therapy and electro-therapy. In the first of these, there is some confusion of identity between opsonins and antibodies. In the discussion of the action of the thyroid principle no mention is made of its effect on metabolism.

The volume, being in its fifth edition, has evidently proven its value.

Practice of Medicine†

THIS volume is a compilation of the work of twenty-six British authorities, edited by Frederick W. Price. It is written as a textbook and should serve its purpose well. It is not intended as a reference volume and because of necessary brevity, it could scarcely be used as such. The editor points out that in his opinion, it is beyond the scope of any one authority, however varied his range of knowledge, to do full justice to the ever widening field of medicine, and it is for this reason that various subjects are discussed by authorities of unusual experience in each field. The book approaches nearer in composition to Osler's Principles and Practice of Medicine than to any other book. It is perhaps intended to take the place of the latter and can best be compared with it. Although of about the same actual size, being printed on thin paper and containing 1753 pages, it is really much larger. The editor has included sections on diseases of the skin, psychological medicine, and tropical diseases. The chief point of difference from Osler is that there are multiple collaborators. This fact has its obvious advantages, but

*Materia Medica for Dentists. Notes on Materia Medica Pharmacology and Therapeutics for Dental Students and Practitioners. By Frank Coleman, M.C., L.R.C.P., Assistant Dental Surgeon St. Bartholomew's Hospital; Dental Surgeon Royal Dental Hospital: Lecturer in Materia Medica to the London School of Dental Surgery. (University of London.) Cloth. Price $3.25. Pp. 308. Fifth edition. Oxford Medical Publications. London. Henry Frowde and Hodder & Stroughton, The Lancet Bldg., 1 Bedford Street, Strand, W. C. 2. 1922.

†A Textbook of the Practice of Medicine, including sections on Diseases of the Skin & Psychological Medicine. By various authors, edited by Frederick W. Price, M.D., F.R.S. (Edin). Senior Physician to the Royal Northern Hospital, and Physician to The National Hospital for Diseases of the Heart, London; formerly Physician and Honorary Pathologist to the Mount Vernon Hospital for Consumption and Diseases of the Chest, and Examiner in Medicine at the University of St. Andrews. Cloth. Oxford Medical Publishers. Pp. 1753. London. Henry Frowde and Hodder & Stoughton, The Lancet Bldg. 1 Bedford Street, Strand, W. C. 2. 1922.

the volume loses the charm of personal touch so evident throughout Osler's Principles and Practice. It will make an excellent textbook.

BOOK NOTICES

COLLOIDS IN BIOLOGY AND MEDICINE, by Prof. H. Bechhold, member of the Royal Institute for Experimental Therapeutics in Frankfort A. M. Authorized Translation from the second German edition with notes and emendations by Jesse G. M. Bullowa A.B., M.D., Assistant Clinical Professor of Medicine, Fordham University; Adjunct Professor of Clinical Medicine, N. Y. Polyclinic School and Hospital; Visiting Physician Riverside Sanitorium; Associate Visiting Physician Willard Parker Hospital, N. Y. City. Cloth. Pp. 464, 54 illustrations. New York. D. Van Nostrand Co. N. Y. 1919.

PHARMACEUTICAL AND FOOD ANALYSIS. A Manual of Standard Methods for the Analysis of oils, fats, waxes, and substances in which they exist; together with allied products, by Azor Thurston, late chemist to the Ohio State Pure Food and Drug Commission. Cloth. Pp. 416. D. Van Nostrand Co. N. Y. 1922.

THE PHYSIOLOGY OF TWINNING. By Horatio Hackett Newman, Professor of Zoology University of Chicago. Cloth. Pp. 230. Chicago. University of Chicago Press. 1923.

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MODERN MICROSCOPY. A handbook for beginners and students. By M. Cross and Martin J. Cole. Lecturer in Histology at Cooke's School of Anatomy. Fifth edition. Revised and rearranged by Herbert F. Angus. Cloth. Chicago Medical Book Co. Chicago, Ill. Pp. 315 with numerous illustrations and diagrams. 1922.

Erratum

In Dr. Mirkin's article in the February issue, page 338, the thirteenth line from the bottom of the page should read:

$$X = \frac{541 \times 579 \times A - 579 \times 563 \times 2.7 \times V}{541(579 - 563)}$$